NOLOGY TRANSFER CONFERENCE • 1988

DATE DUE

ISSN 0840-8440

PROCEEDINGS

TECHNOLOGY TRANSFER CONFERENCE 1988
November 28 and 29, 1988
Royal York Hotel
Toronto, Ontario

SESSION D
ANALYTICAL METHODS

Sponsored by
Research and Technology Branch
Environment Ontario
Ontario, Canada

Introduction

Environment Ontario holds its annual Technology Transfer
Conference to report and publicize the progress made on
Ministry-funded projects. These studies are carried out in
Ontario Universities and by private research organizations
and companies.

The papers presented at Technology Transfer Conference 1988
are published in five volumes of conference Proceedings
corresponding to the following sessions:

        SESSION A: AIR QUALITY RESEARCH
        SESSION B: WATER QUALITY RESEARCH
        SESSION C: LIQUID AND SOLID WASTE RESEARCH
        SESSION D: ANALYTICAL METHODS
        SESSION E: ENVIRONMENTAL ECONOMICS

This volume is comprised of presentations given during
Session D of the conference.

For reference purposes, indices for sessions A,B,C and E
may be found at the back of this volume, listed in alpha-
numeric order.

For further information on any of the papers, please
contact either the authors or the Research and Technology
Branch at (416) 323-4574 or 323-4573.

Acknowledgements

The Conference Committee would like to thank the authors
for their valuable contributions to environmental research
in Ontario.

Disclaimer

The views and ideas expressed in these papers are those of
the authors and do not necessarily reflect the views and
policies of Environment Ontario, nor does mention of trade
names or commercial products constitute endorsement or
recommendation for use.

INDEX

Abstract   **Page**

SESSION D: ANALYTICAL METHODS

Oral Presentations

Abstract	Page

SESSION D: ANALYTICAL METHODS

Oral Presentations

Abstract   Page

SESSION D: ANALYTICAL METHODS

Oral Presentations

SESSION D: ANALYTICAL METHODS

Poster Presentations

KEYNOTE PAPER I

Science-Based Innovation

Science-based innovation is critical in today's global economy to sustain and enhance a nation's prosperity. In seeking to sustain and enhance its prosperity by participating in a growing volume of world trade, large and small economies, face critical problems of adapting their institutions, policies and practices to a radically new environment. Key elements of this environment are that world trade now occurs in a global economy in which the interweaving of science, engineering and technology has acquired the power to transform the comparative advantage and prosperity of nations. With the scale, scope and openness of the international enterprise of science, the transferability of technologies and the mobility of capital, science-based innovation has become a driving force for the technological and corporate change that creates new tradeable goods and services. These conditions are radically different from those of the Industrial Revolution.

In a modern economy the sector which produces tradeable goods and services supported by the first service sector of financial, legal, energy, transportation, communication systems, etc., generates the income that enables a country to invest in the second service sector of education, health care and other personal and social benefits. (Figure 1). In some countries financial institutions have been operating in a manner that hampers the developments in the tradeable goods and services sector.

To participate in the global economy driven by science-based innovation it is essential that, on a national or regional basis, the pyramid of research capacity (in terms of knowledge flow) that leads to tradeable products and services has integrity, that is, that there be a reasonable balance of capacity throughout the pyramid. (Figure 2).

Increasingly science-based innovation requires a strong long-term applied research capacity, particularly in relation to emerging generic technologies, that is industry-based and controlled. This capacity has to be linked to a high quality fundamental research base and a strong market focused development capability.

Large and small countries in different stages of development faces problems in;

    i) achieving structural integrity necessary for science-based innovation suitable for their limited resources of people and money, and

    ii) using their limited resources effectively.

(A)	(B)	(C)
Tradeable Goods and Services	**Service Sector I** • Finance • Legal • Transportation • Communications • Energy • Other	**Service Sector II** • Health • Education • Social Services • Leisure • Recreation
• Income generation • Little direct job creation • Highly skilled jobs	• Some tradeable services • Broad mix of skills • Quality relates to the number of world class operations from a region	• Dependant on income from (A) • Sector of job creation • Low to highly skilled jobs • Influences quality of (A) and (B)

Figure 1: A Simple Model of the Economy

In today's global economy it is important to understand the relationship between innovation in the production of tradeable goods and services and the generation of income. A simple model in terms of stating the key issues is given in Figure 1. This model segments the economy into three blocks labeled (A) Tradeable Goods and Services, (B) Service Sector I and (C) Service Sector II. The major source of income which sustains our standard of living comes from sector (A) Tradeable Goods and Services. Canada's current standard of living requires very substantial volumes of trade into world markets. In the globally competitive market of today a nation must be concerned with maintaining and enhancing the environment it creates for business and industry that can innovate in the production of tradeable goods and services. To function effectively, such enterprises require a high quality service sector, namely (B) Service Sector I, comprising such services as finance, legal, energy, construction, communications, transportation, distribution. It is the combination of this business service sector (which produces some tradeable services) and the sector directly producing tradeable goods and services which generate the primary income of a region.

It is the income generated by the foregoing activities which allows the expansion of (C) Service Sector II that is concerned with personal and social services. The social service sector includes health care, education, community services, leisure and recreational activities. Our capacity to sustain and improve the services and opportunities depends on the capacity of sectors (A) and (B) to generate the necessary income.

2

The Science-Based Pyramid of Research

Category of Research

Developmental

Competitive or
Short-term Applied

Pre-competitive
or Long-term
Applied

Basic

Product or Service
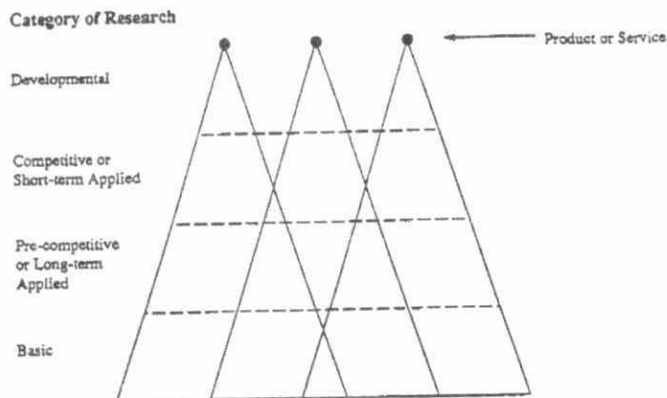
Figure 2

Research as an element related to the overall process of innovation, can be broken down into three primary components that must be linked together to be effective:

1. Basic or fundamental research which is usually characterized by the researchers' primary objective being the generation of new knowledge and understanding about man and the world around us. This research is long-term (usually on a time-scale of 10 years or more), and has a high level of uncertainty in terms of what the results will be. In Western culture, basic research is primarily university-based and seldom results in knowledge that is of immediate commercial value. The knowledge gained from such research is rapidly and widely distributed to scientists throughout the world through publication in scientific journals. Because the results from this research are, or have been, considered a public good, this type of research has been financed primarily by the public sector and private benefactors. Increasingly, however, when knowledge contributed by basic research is critical for new product development, industries are becoming involved in basic research (OECD, 1987).

3

2. **Applied research:** In most countries, applied research is mainly carried out in industrial or government laboratories, but in some countries, there is substantial university involvement with respect to longer term research, particularly in schools of engineering, medicine and management. Applied research has a strategic target and attempts:

   - to extend the scope of understanding of materials and processes,

   - to determine how the accumulated knowledge from basic research, extend where necessary by focused specialized research, can be used to develop a potential new product or services, or

   - to determine how to modify and improve the performance of existing products or services to sustain their marketability.

   Applied research which is medium to long-term (on a time scale of three to ten years), also has a significant level of uncertainty, but because it is targeted, there is a probability that there will be economic benefit. The means for the financing of this research vary. In some sectors such as the pharmaceutical and chemical fields, the research is largely funded by the private sector primarily through the benefits from patent protection, whereas in fields such as aircraft and electronics, there has been a mixture of public and private financing. In some cases a monopoly position (e.g. AT&T and Bell Laboratories) has encouraged the funding of longer-term applied research, but there are few examples of the private sector being able to finance longer term applied research wholly from its own resources unless there is effective patent protection or the business has a monopoly position.

   It is common in some sectors to associate the processes of engineering design and development of a product or service as discussed above, with the term development or developmental research.

3. **Developmental research** is research that:

   - makes use of the fruits of applied research specifically to create a new marketable product or service, or

   - improves, through a series of small steps of innovation based on state-of-the-art knowledge, an already existing product or service, or

   - enhances the ease of production of a product or the provision of a service.

   This type of research has the least uncertainty, is carried out on a time scale of less than three years, and has the highest probability of economic benefit. Developmental research is mainly financed by the private sector, although there are exceptions in which there has been substantial public financing.

4

The foregoing categories of research can be represented by the pyramids shown in Figure 2. At the narrow peak of each pyramid is a product or service, a specific artifact of technology designed to perform a particular function in a market. From its peak each pyramid expands through the three primary categories of research to a broad base in basic research. The category "applied research" has been segmented into two slices labeled competitive (short-term applied) and pre-competitive (long-term applied). Competitive applied research is that which has direct proprietary value to the business. Pre-competitive applied research is that which is generally useful in sectors of industry (This research is often concerned with what can be called generic technology). The relative width of each slice across the pyramid suggests the range of generality of the knowledge associated with it. The overlapping of parts of the pyramids indicates that as one reaches towards the scientific roots pertinent to the development of a particular product, the knowledge base becomes relevant to a range of products. Indeed, the essence of basic science is that it seeks for general principles of understanding within particular circumstances of study, whereas engineering, through the technology it creates, seeks to realize a particular operational function in a market within the domain of possibilities bounded by science.

Science-based innovation then is innovation in which the realization of an effective and competitive product or service utilizes, through the focusing processes of the pyramid of research, the full range of scientific and engineering understanding pertinent to the function of the product or service in the marketplace.

The classification of levels of research in the research pyramid of Figure 2 is based on the diverse literature on innovation. Its pertinence for older, large-scale, science-based industries is clear. However, a key point today is that the research pyramid is relevant to all industry participating in the global economy of science-based innovation.

Copies of "INNOVATION AND CANADA'S PROSPERITY: THE TRANSFORMING POWER OF SCIENCE, ENGINEERING AND TECHNOLOGY" may be obtained by filling out the attached form.

KEYNOTE PAPER II

DERIVING BENEFITS FROM ENVIRONMENTAL RESEARCH

Stuart L. Smith, M.D.
President
RockCliffe Research and Technology Inc.
November 1988

As difficult as research can be, it is still more difficult to apply it swiftly for economic or social benefit. In addition to the usual obstacles to technology transfer, environmental research faces additional ones of a political nature. It behooves us to know a great deal more about how research is transformed into practical benefits and how environmental research in particular can be more rapidly applied. The improvement of the environment is an area where, with appropriate policies, economic and social benefits occur simultaneously.

In supporting research activities, we cannot take for granted that application will naturally follow any improvement in knowledge. More attention needs to be paid to the incentive structures of research organizations, the relationship to our industrial sector, and the interaction with political decision-making. By acting now in some specific areas, we can help guarantee that today's research will produce timely and tangible results.

SESSION D
ANALYTICAL METHODS
Oral Presentations
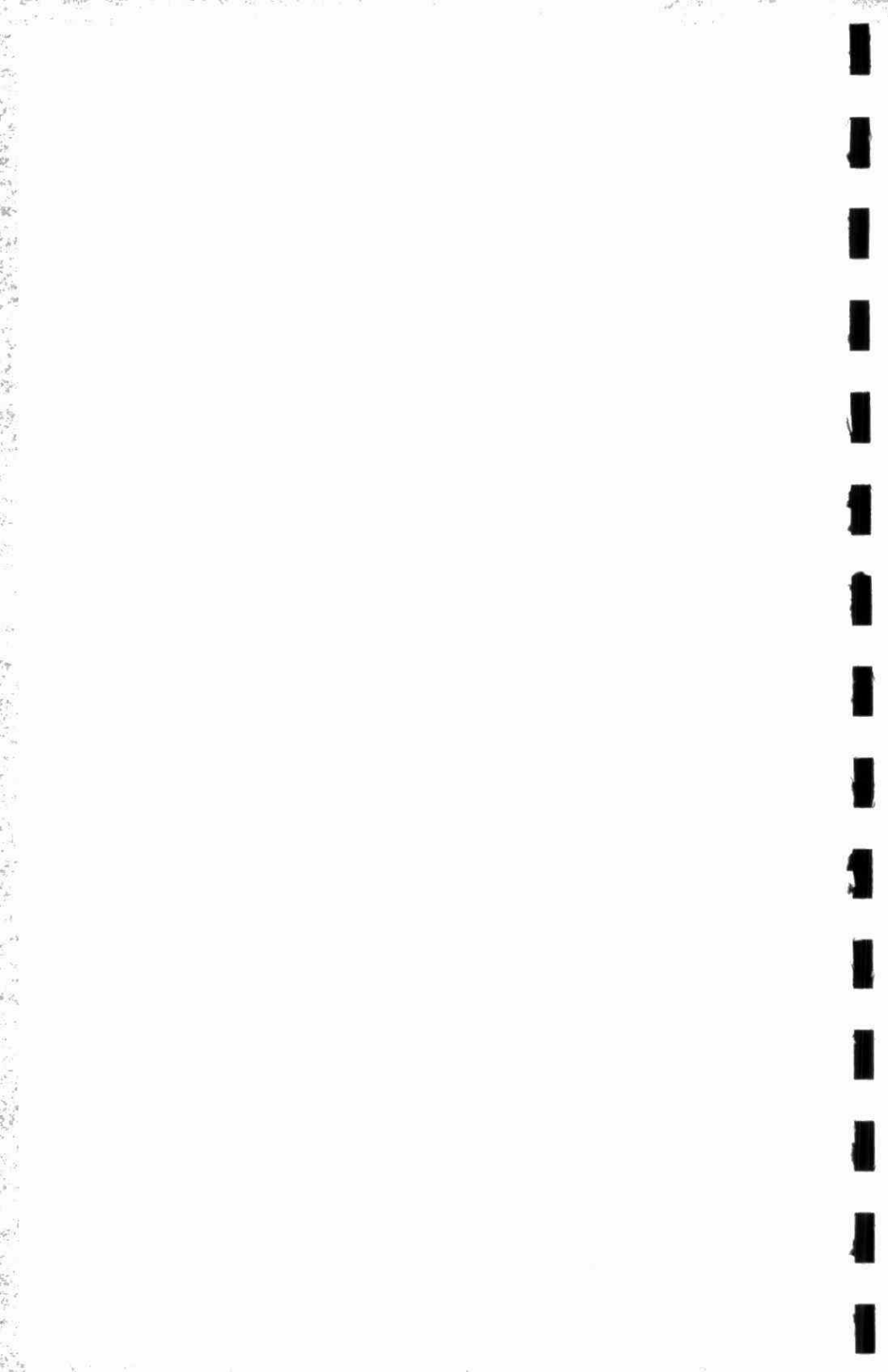
ENVIRONMENT ONTARIO PAPER

ANALYTICAL METHODS DEVELOPMENT RESEARCH

Ray Clement
Laboratory Services Branch
Environment Ontario

Virtually every aspect of environmental research requires sample analysis. Sound methodologies are required for studies of a wide range of organic and inorganic contaminants of drinking and surface waters, groundwater, landfill leachates, ambient air, stack emissions, hazardous wastes, sewage and pulp and paper sludges, and biological systems. In addition to developing new methods for performing qualitative and quantitative determinations at trace levels in complex matrices, there is a constant need to improve existing methods to allow for analysis of more samples, better and faster. Specific research needs cover the gamut of analysis steps from sampling to analyte identification, quantification, and data interpretation.

Research is needed in the analytical area to determine the effect of sampling protocols on analytical data, especially for microbiological parameters. Field methods for pre-concentration of viruses and integrative biological tests to identify problem areas in air, soil and water are required. Rugged field collection methods for large-volume aqueous samples are needed using sorbent cartridges or other means. Associated sample preservation, shipping and storage protocols to ensure analyte stability are equally important. Techniques for odorous compounds in water and air and non-open collection methods to avoid atmospheric contamination or degassing of groundwaters/landfill leachates are of special importance. A validated, tiered testing protocol for groundwater contaminants is also required, in addition to improved methods for semi-volatile organics and trace metals in ambient air. Cannister sampling methods, plume dispersion models and improved prediction of precipitation events are all required to improve air and atmospheric deposition studies.

Samples often must be sub-aliquoted for analysis of multiple analytes and round-robin investigations. Effects of grinding, slicing, blending and other mixing and homogenization procedures on sample integrity need to be performed. Determination of analyte losses and the potential for artifact introduction and sample cross-contamination are especially important. Procedures to ensure the homogeneity of aqueous samples containing suspended particulates during sub-aliquoting also require investigation. Laboratory protocols for recovery and enumeration of pathogenic organisms need to be developed.

An ongoing need is to improve methods for the unambiguous identification of a broad range of organics in complex samples such as pulp and paper and sewage sludges. Methods to be investigated include advanced GC-MS techniques, FTIR, computer library matching, and other chemometrics techniques. Isolation

and concentration methods to obtain large quantities of
unidentified analytes are required to employ other spectroscopic
techniques such as NMR. Improved chromatographic separation and
HPLC fractionation techniques to simplify complex organic mixtures
prior to mass spectrometry detection are also needed. Rapid
screening methods for trace metals in aqueous samples by using
ICP-MS, GC-microwave plasma emission, or biological on-line
monitoring are also of interest.

Microbiological identification enhancements are required for
E. Coli and group D. enterococci in water and sediments, as well
as modification of such methods for the detection of other
pathogens such as Salmonella and Compylobacteria. Similar methods
to identify genotoxic compounds or conditions in the environment
are needed. An ongoing problem is how to relate carcinogenicity
and genotoxicity test responses to human health effects.

Improved quantification of target analytes is an ongoing
research need. Development of novel GC detectors or improving the
selectivity of conventional detectors is one approach to be
considered. Development of improved cleanup techniques to isolate
chemicals of concern also needs to be studied. the unambiguous
determination of all 2,3,7,8-substituted dioxin/furan congeners is
of special interest. To improve the quantitative analysis of
selected target analytes in specific matrices, research is needed
to develop validated analytical standards and SRMs.

For all of the preceeding studies improvement of sample
throughput and the analytical precision and accuracy are desired.
Approaches include chemometric techniques for data analysis
including development of expert systems for data analysis, QA/QC,
and troubleshooting analytical instruments. Other enhancements
can be obtained through the use of robotics and development of new
sample preparation methods more suited to automation. One area of
special interest is the extraction/concentration of samples using
supercritical fluids.

In addition to all of the above, some critical Ministry
programs have special requirements. Of particular importance are
laboratory techniques to assess the leaching potential of specific
wastes for hazardous waste classification. Methods for complex
industrial effluents for the MISA program are also important.
Specific research is required to evaluate interferences for the
determination of inorganics in MISA effluents, to develop improved
analyte detectability by matrix modification, and to improve and
validate methods for quantifying volatiles associated with liquid
and solid fractions of sludges. Special problems have been
encountered with the complex samples derived from pulp and paper
industries, for which research is needed for sediment and biota
tissue analysis of resin and fatty acids and speciated phenols.
In addition, methods for flowthrough field analysis of pulp and
paper effluents to determine the impact of mill discharges are
required. Research is also needed to develop methods for the
source tracking of pollutants in air and water. Chemometric
methods are needed to compare organic/inorganic analyte profiles
to identify generic sample types, to identify specific sources of
such groups of compounds as PCBs, dioxin/furans and toxaphene by
studying their congener/isomer patterns, and by studying isotope
ratios of elements such as lead.

13

D1

ADVANCES IN THE IDENTIFICATION
AND ANALYSIS OF ORGANIC POLLUTANTS IN WATER

R. O. Kagel   J. E. Norris
Dow Chemical U.S.A.   BMC Converse

In the early 1970's the interface between the regulatory and
analytical communities can be best described as chaotic. The
field was dominated as much by lawyers as it was by scientists.
The limit of detection and the regulatory limit were often
confused and fuzzy. In the lawyer's world below the regulatory
limit one was in compliance; above the limit one was in
violation. The limit itself was infinitesimal in thickness.
Conformance or non-conformance was limited only by the number
of figures your computer printed out. The laws of probability
were repealed insofar as recognizing that legitimate scientific
factors introduce uncertainties into measurements. Instead all
uncertainty was assigned to human error. This made laws easy to
write and comprehend but scientifically untenable to enforce.

The American Chemical Society took a positive step in the late
1970's towards introducing some order to the field of issuing
"Guidelines for Data Acquisition and Data Quality Evaluation in
Environmental Chemistry" which defined limits of detection and
limits of quantification. These guidelines were followed shortly
by the Environmental Protection Agency's Method Detection Limits
(MDL's). EPA's October 1984 promulgation of its Clean Water
Act 40 CFR 136 Analytical Methods for Priority Pollutants
incorporated the concept of Method Detection Limits. Since these
analytical methods formed the nucleus for the SW-846 methods to
be used by EPA's Office of Solid Waste (RCRA/CERCLA), the MDL
concept entered this regulatory scheme also. Finally, EPA has
defined a concept of Practical Quantitation Level (PQL) for use
with the so-called 500-series methods prescribed under Safe
Drinking Water Act.

These various measurements of method performance, their realistic
limitations and the role of statistical uncertainties in PPB and
PPT analyses will be discussed in this paper.

D2

ADAPTATION OF WATER PRECONCENTRATION TECHNIQUES DEVELOPED FOR PCDD ANALYSIS TO OTHER TARGET ORGANIC POLLUTANTS. E. Dowdall*, B.R. Hollebone, L.B. Brownlee, C. Shewchuk, Carleton University, Ottawa, Ontario, K1S 5B6

Introduction

The analysis of water for organic pollutants involves preconcentration, isolation and detection steps which must be coordinated to produce acceptable detectivity and sensitivity in relevant concentration ranges. In this project, the development of a preconcentration apparatus, designed for isolation of dioxins, is being extended to a wider range of chlorinated hydrocarbons and pesticides in natural and treated waters. The modifications to the sampler are minor, requiring only the optimization of filtration cylinders and adsorption media. The isolation steps, however, require considerable development beyond the laborious and highly specialized procedures developed for the dioxins.

There are two major areas which require attention. First, the isolation procedures used for dioxins must be altered because they were designed to remove many of the eluted or extracted organic pollutants from detection which are now of interest. Secondly, the GC/MS detection procedures used for dioxins must now be broadened to observe the much larger number of compounds of interest.

The developments associated with adaptation of the automatic

17

preconcentration and traditional or supercritical isolation of compounds for detection are the subjects of the present research. The problems of broadening detection capabilities of GC/MS library identification routines require further attention and are beyond the scope of this paper.

Experimental Design

Since the project is in its early stages, the experimental work is still in progress. Two directions of research are being undertaken.

(1) Construction of Preconcentration Sampler

The sampler is an improved version of one developed for dioxin preconcentration which is currently under test by the Ministry of Environment. A schematic of water flow through the sampler is illustrated in figure 1. The overall design and placement of components is shown in figures 2 and 3.

The system is designed to provide two identical filtered and adsorbed samples of known volumes from a single source, without the introduction of external contamination. A water reservoir isolates the sampling unit from the water source by providing a physical break in the water flow. This sealed vessel only permits water flow in one direction through an electronically operated input valve. The internal air pressure is controlled

18

through electronically operated valves, and extraneous air is exchanged through a charcoal filter.

The water is pumped past an optional injection port for controlled input of standard solutions, then into a helical mixing tube, and split into two sampling streams. These two streams pass through one or more removable filter chambers, as required, which contain tubular filters optimized for the particular water source. Each stream passes through a graphite gear pump and is brought to an operating pressure of about 20 psi. The streams then pass through visual flow meters and pressure gauges, and then to air release valves which permit the exclusion of air bubbles. This deaerated water is then pumped through removable XAD-2 resin adsorption columns. The two streams are recombined and pass through an electronic flow sensor which sends a signal to a digital flow meter to record the total flow rate. It is then exhausted through a final charcoal filter.

The filters and columns are prepared in the laboratory and can be installed in the sampler at the field site. Prior to each run, the total desired sample volume is established using a batch controller. The water system is first purged with source water, bypassing the filter and columns, and is then switched to sampling mode to be put under control of the batch computer. The flow rates are equalized manually by adjustment of the pump speed controls until the individual flow meters read identical values and the digital flow meter reads the desired total flow rate. The batch controller integrates the total volume and when the

preset value is achieved the system shuts off automatically. Automatic shutdown also occurs if pressure in the sampler exceeds 50 psi or falls below 3 psi, or if the housing to the filter or adsorption system is opened.

When sampling is completed, a recording of the flow rate throughout the run is obtained from a chart recorder. The filters and adsorption columns, which are isolated with self contained valves, are removed from the apparatus for transportation to the analytical laboratory.

The entire apparatus is housed in a self sealing case with the dimensions 1.5 m high x 0.5 m wide x 0.5 m deep, and weighing 75 kg. It is mounted on self contained wheels so it can be transported manually from site to site. Apart from the necessary connection to the water source and installation of filter chambers and adsorption columns, all functions are electronically controlled to eliminate dependence on manual operation.

(2) Sample Isolation

Samples received in filtration chambers and adsorption columns must be eluted and treated in the laboratory to isolate the compounds of interest. The two basic approaches to the processing of these samples are the traditional extraction with organic solvents and the more recent method of extraction using supercritical fluids.

The organic solvent method requires the use of a volatile solvent to produce a solution of soluble organic contaminants

from the water sample. This normally includes a dominant fraction of humic and fulvic acids, or even chlorinated versions of these, from treated waters. If ultratrace levels (parts per quadrillion) of the target analytes are to be analyzed, the humic acids must be removed. A common way to achieve this is by oxidation with concentrated sulphuric acid at room temperature[1]. Fractionation can then be undertaken by passing the solution through a series of adsorbing columns. These eluants are then evaporated for final GC/MS detection.

It is recognized that this method is complex and possibly inaccurate. The acid oxidation treatment is capable of destroying compounds of interest while removing the humic fraction. The subsequent fractionation techniques can have low elution recoveries as has been observed in research on the octachlorodibenzo-p-dioxin congener. Thus, large recovery correction factors are common in analytical data.

A further drawback is the complexity of automation which might be attempted in order to avoid manual labour, time and expense. The number of steps and reagents makes automation very difficult to control. The corrosive or solubilizing nature of the reagents requires an expensive apparatus which may be subject to decay.

A highly viable alternative is the extraction by supercritical fluids, developed in the food and chemical processing industries. Essentially, the samples in their chambers may be dried under vacuum and then extracted

21

automatically under pressure by liquid CO_2 at room temperature on a single pressure system[2] (see figure 4).

Aside from reducing opportunities for contamination and greatly simplifying sample handling, the process also eliminates many of the chemical isolation steps. This is because liquid CO_2 is capable of extracting small hydrophobic molecules from XAD-2 with 95% recovery at room temperature and operating under a pressure of 2000 psi, without solubilizing the polymeric humic acids. After flashing off liquid CO_2, the sample is delivered as a solid deposit of the compounds of interest only. The oxidation step is unnecessary and the only question remaining is the number of fractionation steps required to permit GC/MS detection and identification.

In the present apparatus, the adsorption columns are capable of direct attachment to the pressure line. The filter chambers, however, cannot sustain the pressure. They will be installed in high pressure chambers and held in place by a hydraulic ram. The filter chamber will then be pressurized simultaneously outside and inside, the inside stream operating as the elution system. As a result, both types of chamber can be extracted within 10 to 15 minutes, producing dried samples ready for dissolution in any chosen solvent, followed either by direct GC/MS injection or fractionation prior to injection.

References:

1/ G.L. LeBel, D.T. Williams, J.J. Ryan and B.P.-Y. Lau,
    "Chlorinated Dioxins and Dibenzofurans in Perspective" (C.
    Rappe et. al. eds.) (Lewis Publishers, Michigan,
    1986) 329-341

2/ M.A. McHugh and V.J. Krukonis, "Supercritical Fluid
    Extraction", Butterworth Publishers, MA, U.S., 1986.

FIGURE 1
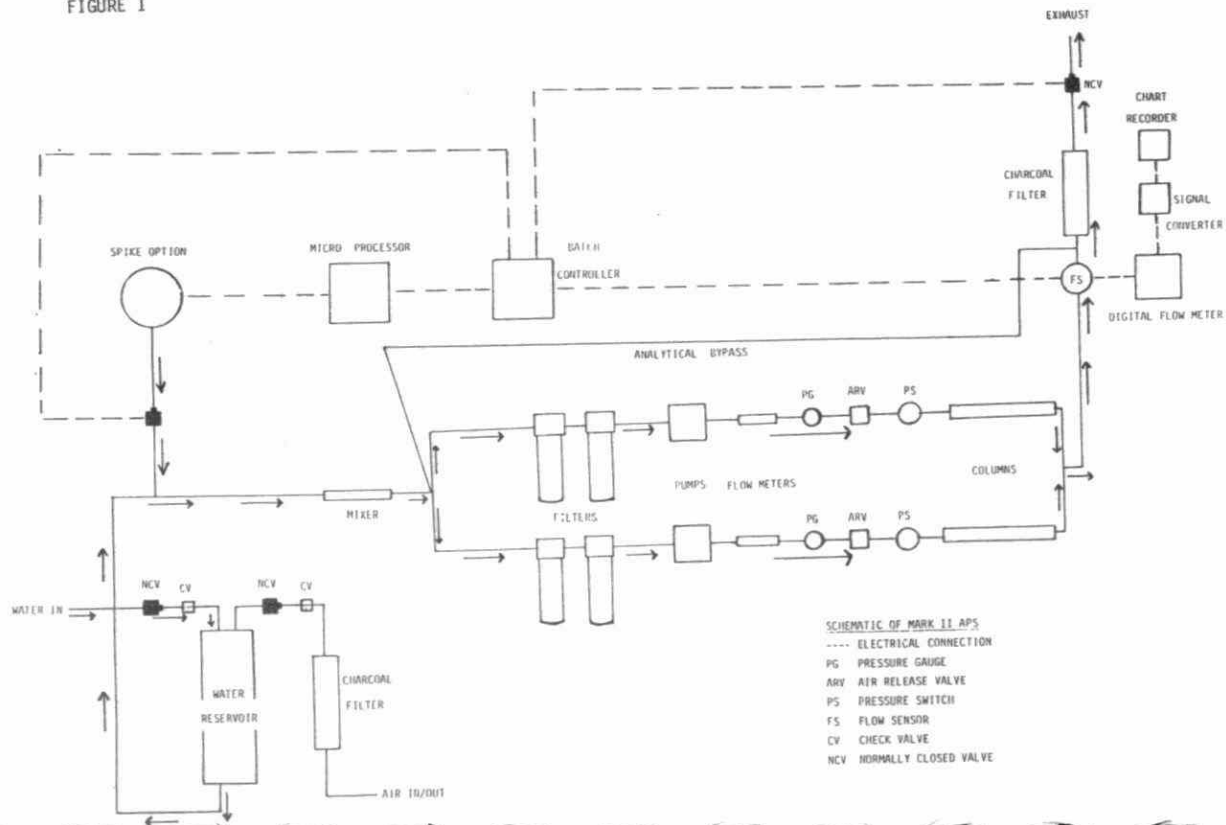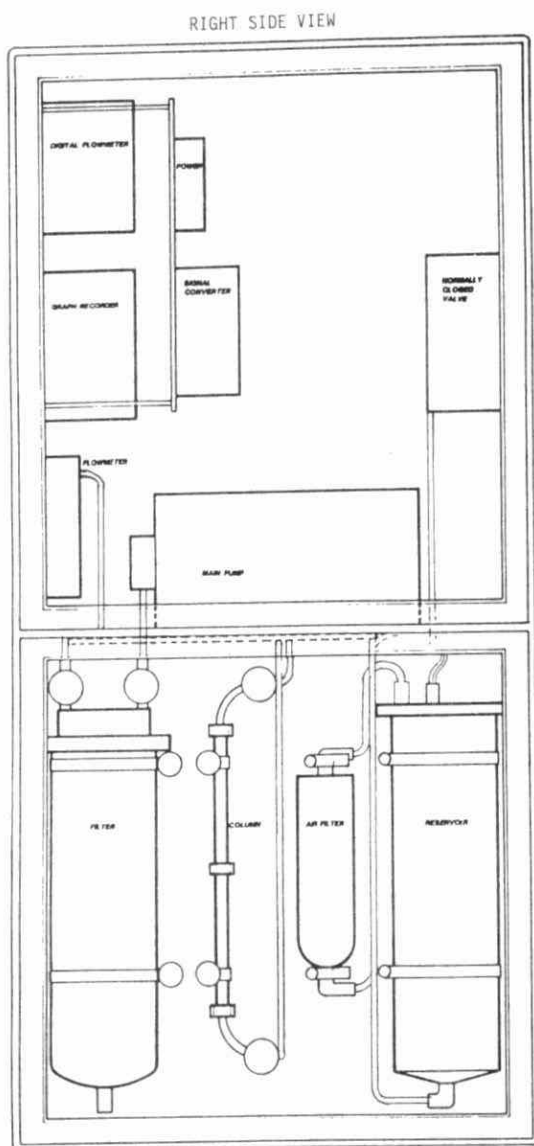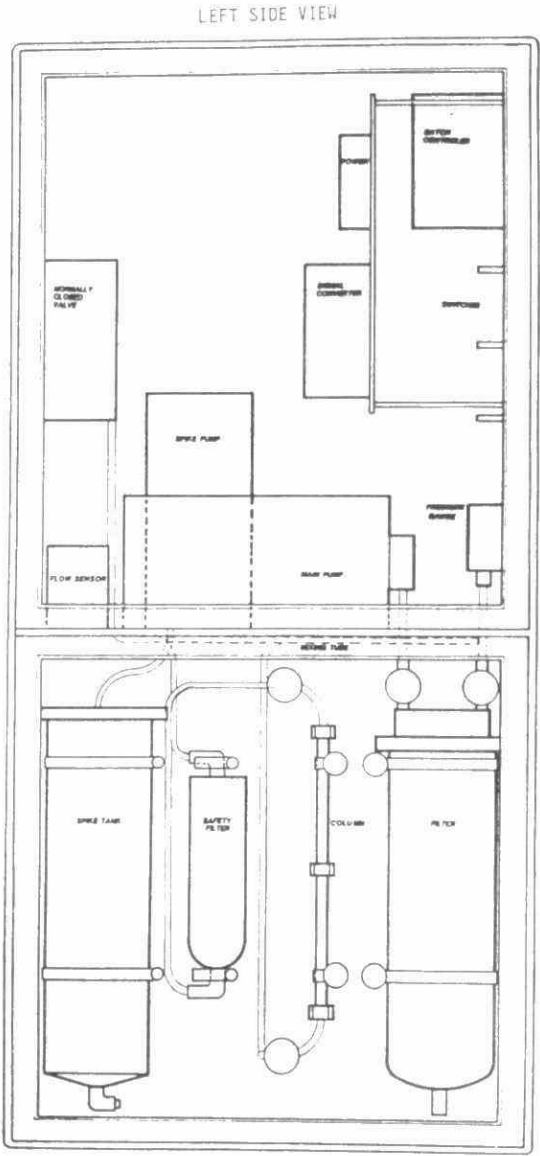
EXHAUST

NCV

CHART
RECORDER

CHARCOAL
FILTER

SIGNAL
CONVERTER

SPIKE OPTION

MICRO PROCESSOR

BATCH
CONTROLLER

FS

DIGITAL FLOW METER

ANALYTICAL BYPASS

PG   ARV   PS

PUMPS   FLOW METERS

COLUMNS

MIXER

FILTERS

PG   ARV   PS

WATER IN

NCV   CV   NCV   CV

WATER

RESERVOIR

CHARCOAL
FILTER

AIR IN/OUT

SCHEMATIC OF MARK II APS
---- ELECTRICAL CONNECTION
PG   PRESSURE GAUGE
ARV   AIR RELEASE VALVE
PS   PRESSURE SWITCH
FS   FLOW SENSOR
CV   CHECK VALVE
NCV   NORMALLY CLOSED VALVE

FIGURE 2

RIGHT SIDE VIEW

LEFT SIDE VIEW

FIGURE 3

FIGURE 4

System Schematic

Relief Valve

Constant Pressure CO2 Pump

Check Valve

Shut-Off

Quick-Connect

Extractor

Temperature Control

Quick-Connect

Shut-Off

Precision Orifice

to sample collection or on-line analysis

Sample Collection

from extractor

vent to charcoal filter

sealed sample vial

temperature control
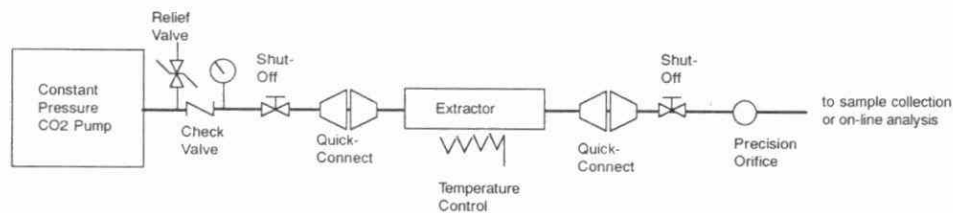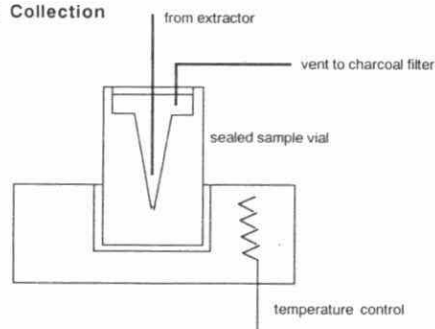
D3

THE PURPOSE AND SIGNIFICANCE OF ULTRATRACE ANALYSIS OF DIBENZO-P-DIOXINS: THE CONCEPT OF RISK.   L. Brownlee* and B.R. Hollebone, Chemistry Department, Carleton University,   Ottawa, Ontario, K1S 5B6.

INTRODUCTION

The   ultimate   goal   of   the   toxicology   disipline   is   to identify,   define   and   respond   to   the health risks of chemical agents.   The advent of chemicals in the environment is relatively recent, beginning with the industrialization of Europe.   Since the second world war the release of chemicals has increased alarmingly, the benefits of these chemicals in short-term growth, medical advancements and control of the environment through pesticides and fertilizers overshadowing any long term negative effects. Only in the last 25 years have government agencies been forced to acknowledge the harmful effects of many industrial chemicals.   The onus has been on these agencies to accumulate the data necessary to control the chemical industry.

Government resources, in both manpower and money have gone into environmental monitoring and health assessment studies in order to define the risk of chemicals already in the environment. Using this knowledge, effective legislation to control future chemical release into the environment has begun only in the last decade. Much work is left to be done.

The health risk of a chemical can be defined as follows:

$$\text{TOTAL RISK} = \text{SUM OF ALL EXPOSURES} \times \text{SUM OF ALL HAZARDS} \quad (i)$$

in which, Exposure has the units, Dose and Hazard has the units, Response/Dose. Inserting these units into the equation;

$$\text{RISK} = \text{DOSE} \times \text{RESPONSE} / \text{DOSE} \quad (ii)$$

which has the dimension of a % change in behaviour. This is a very broad equation when considered in terms of the total environment.   The Toxicologist must sift through all the variables of this equation to identify the key exposure and resulting hazard to determine the ultimate risk to man.

Exposure:

Chemicals are released into the environment by three routes; by air through waste emission; on land through application; and into water via effluent waste.   Much of the air and land pollution will precipitate or leach into the water system. From these primary routes, pollutants move into the human food supply through meat, produce and drinking water.   Quantification of exposure is an analytical problem. Routine monitoring studies of

air, land and water identify problem areas and pinpoint sources of pollution. Residue studies of plants and animals determine the effect these chemicals have on the food chain. These data are used to predict the chemical exposure of man.

A great deal of research into the measurement of exposure has been done. Many research groups including our own are presenting "state of the art" methodologies at this conference that will aid toward better understanding of chemical exposure in Canada. Better analytical and monitoring techniques will allow better identification of populations that are "at risk" from environmental exposure.

Hazard:

Two fundamental problems of Risk Assessment are the determination of at what levels of environmental exposure a chemical becomes a risk and what the consequences of that risk are. This encompasses the hazard part of the risk equation.

The traditional approach to determining the hazard of a chemical is to study the toxic symptoms of laboratory animals at the medical level in response to a particular chemical dose and to try and correlate this dose to biochemical derangements.

In many studies the toxic medical responses of test animal to chemicals are the development of cancer or death. Detailed autopsies and extensive biochemical testing complement these studies to determine subcellular perturbations that could have resulted in the toxic medical symptoms. Linking these biochemical symptoms to the original chemical dose is a very labour intensive, complex and expensive mandate. Considering all the chemicals in the environment, a more streamlined and cost effective approach to screening potentially toxic chemicals is needed.

From the literature it is well understood that the hepatic Mixed Function Oxidase system is involved in drug tolerance and the activation of chemicals into carcinogens. Ten years ago these laboratories undertook to reverse the traditional approach to determine chemical hazard by studying the derangements of this system and how they relate to the ultimate toxic system. Our objective was to understand the behaviour of the MFO system in response to in vivo and in vitro chemical exposure and with this information develop a screening procedure for exposure to toxic chemicals. This was done by identifying normal and abnormal biochemistry and correlating perturbations with the overall health of the animal.

MODEL OF RESPONSE OF DEFENSIVE ENZYME SYSTEMS

The Mixed Function Oxidase system, also known as Cytochrome

P-450, is a group of ubiquitous hemoproteins found in microorganisms, plants and animals, whose main purpose is to oxygenate lipophilic, non-nutrient compounds in order to eliminate them from the host (1). From a toxicology point of view, the hepatic microsomal P-450 found in birds and mammals is most interesting. As well as metabolizing certain endogenous compounds, hepatic P-450 detoxifies drugs and other xenobiotics. Most of the time this system works very efficiently to maintain biochemical homeostasis. When confronted with an excess of chemical, i.e. administration of a therapeutic drug, this system can function too efficiently, creating a tolerance and decreasing the effectiveness of the drug. In other cases this process may function in error and instead of detoxifying the chemical agent, the enzyme may convert the substrate into a mutagenic and/or carcinogenic form. It is this characteristic that most interests toxicologists.

Cytochrome P-450 is a large globular octameric protein embedded into two dimensional lipid film called the endoplasimc reticulum (ER) of the cell. In hepatic ER, NADPH-Cytochrome P-450 reductase is also present. This enzyme system catalyzes electron transfer from NADPH to the cytochrome during the oxygen metabolizing process (2). In the traditional model (3), cytochrome P-450 is described as being a rosette surrounding the reductase enzyme. In a second model, the reductase moves like a ship through the P-450 molecules, simulating a sea of rocks. The stoichiometry of P-450 molecules is between 10:1 and 30:1 (3). In this model, the active site of P-450 is an iron protoporphyrin IX moiety that is located in a large, relatively open hydrophobic pocket in the surface of the protein (3) and the xenobiotic molecule is bound directly from the aqueous cytosol of the cell.

The inducibility of Cytochrome P-450:

In the absence of xenobiotics, Cytochrome P-450 in barrier and disposal tissues is at low levels. The introduction of substrates causes synthesis of new protein within minutes. This induction of the MFO system involves simultaneous synthesis of all other components and the proliferation of new endoplasmic reticulum (4). Cytochrome P-450 is in fact, the generalized name given to a group of isozymes embedded in this new protein. Traditionally, induction of P-450 is defined as an increase in enzyme concentration or an increase in the activity of a specific substrate. When the substrate concentration in the liver is reduced, the enzyme is catabolized back to basal levels. The stimulation of the Cytochrome P-450 system results specifically in measurable increases in enzyme activity, concentration of enzyme, proliferation of endoplasmic reticulum and liver weight. This inducibility of Cytochrome P-450 plays an important role in drug tolerance, which is traditionally exemplified by barbituates such as phenobarbitol and hexabarbitol. This was the

characteristic that first drew attention to the liver metabolizing system.

The general form of Cytochrome P-450 may in fact be a mixture of several isoforms present together. Indeed, specific chemicals will induce immunochemically distinct isozymes of P-450. There are now 8-11 distinct forms of induced Cytochrome P-450 isolated.

The best documented isozyme is induced by polyaromatic hydrocarbons (PAH's) found distinctly in the left lobe of the liver, and is commonly called Cytochrome P448 (5). This isozyme was first implicated in cancer research by Conney and his associates in the 1950's (4). They found that when rats were treated with PAH's such as benzpyrene (BP) and 3-methylcolanthrene (3-MC), the ability of the hepatic microsomes to hydroxylate BP increased up to 50 fold with little proliferation of ER or increase in P-450 concentration. As PAH's were known carcinogens i.e. found in smog, car exhaust and cigarette smoke, this unusually high activity was implicated in the carcinogenic mechanism.

In 1963 Fouts discovered that Cytochrome P-450 was very sensitive to chlorinated compounds when his animal room was fumigated with chlordane in the middle of a chronic feeding study (6). Although the original experiment was ruined, this discovery initiated a whole new field of Cytochrome P-450 chemistry. This work was extended to other chlorinated environmental compounds such as DDT, aldrin, dieldrin and Aroclor. In the early 1970's this work was extended to 2,3,7,8 tetrachlorodibenzo-p dioxin (TCDD), a by product of 2,4,5 Trichlorophenoxyacetic acid (2,4,5-T) production that was becoming very controversial due to industrial accidents and Agent Orange exposure in Vietnam.

THE EXPERIMENTAL APPROACH OF THESE LABORATORIES

Traditionally, the mechanism of the Cytochrome P-450 system has been studied using biochemical techniques. In early work, chemicals administered in vivo to the test animals were chosen for their known effects on the MFO system. Chemicals used in in vitro biochemical assays were chosen for their metabolite detection characteristics. In the last decade, many sophisticated biochemical techniques such as immunochemistry have advanced the field. Using this approach, some excellent work in the comparison of induction between species and in defining the induction of isozymes has resulted.

After the MFO system was found to respond to chlorinated compounds, environmental scientists became interested in using the enzyme as an environmental probe. Although some toxicological information using existing biochemical methods was possible, these methods were too qualitative to determine the

biological hazard of a chemical without artificially modifying the MFO system. These laboratories have studied the MFO system using a more toxicological approach by studying the direct response of the MFO system to chemicals selected for specific chemical properties. The experiments were designed to study the in vivo and in vitro toxicities of chemical probes on the behaviour of this defensive enzyme system found in the microsomal fraction of rat liver.

The Total Hepatic Induction (THI) index:

The first step in our approach was to quantitate the biochemical response of the MFO system to these chemical probes. The Cytochrome P-450 system is a very flexable system and is known to have an array of biological responses that it can use in order to adapt to a xenobiotic. The most evident basic responses of the P-450 system itself include an increase in biotransform enzyme activity, increase in enzyme concentration, a proliferation of endoplasmic reticulum and an enlargement of the liver (1). A quantitative system to monitor these responses would give an indication of the ability of the organism to cope with specific chemical stress and therefore give and indication of the "hazard" presented by that chemical. Using these responses, the Total Hepatic Induction (THI) index was developed to monitor the total liver microsomal response to inducer (7). This index was designed to quantitate, rather then replace detailed biochemical studies by providing a simplified picture of a complicated response.

This index was based on the assumption that the Mixed Function Oxidase (MFO) system was the rate limiting step in the metabolism and excretion of lipophilic xenobiotics in the body and that the steps preceding and following hydroxylation do not inhibit the process.

The total experimental index can be expressed as follows:

$$THI_{experimental} = \frac{\text{Enzyme activity}}{\text{[Cytochrome P-450]}}$$

$$X \quad \frac{\text{[Cytochrome P-450]}}{\text{[Microsomal Protein]}}$$

$$X \quad \frac{\text{[Microsomal Protein]}}{\text{Liver Weight}}$$

$$X \quad \frac{\text{Liver Weight}}{\text{Animal weight}}$$

33

$$= \frac{\text{Enzyme Activity}}{\text{Animal Weight}} \qquad \text{(iii)}$$

Each component represents a change in state affected by the xenobiotic, hence, all are presented relative to controls. The dose response of each parameter is dependent upon the detoxification requirements and each parameter tends to compensate for any deficiency in response of another parameter. The THI index can be used to either provide a basis of comparing the total hepatic response of an individual to an xenobiotic or to compare responses between species to environmental contaminants.

This THI index has been used in our laboratories since 1982 on a routine basis. We have often found that a statistically significant difference from control of one parameter i.e. an increase in enzyme activity per P-450 concentration can be countered by an opposite change in a second parameter i.e. a decrease in the concentration of P-450 per unit protein. The final THI parameter was not statistically different from control animals. Statistically different THI indexes occurred when the response of an isozyme such as Cytochrome P-448 was evoked. This compensation mechanism is not normally seen when using typical biochemical techniques and suggests that the MFO system has a chemical response system that is more subtle then the well known isozyme response.

The MFO adaptation to C-H bond strength:

The second step in our toxicological approach was to examine the possible mechanisms of control at the active site of the enzyme system (8).

In most biochemical processes the enzymes are highly specialized to the geometry of one or a small number of substrates. For these particular compounds, the barrier to reaction is reduced by very specific control of the entropy of the reaction transition state (ΔS^*). However, like all catalysts, enzymes may also assist the chemical change by providing sufficient energy to break old bonds or make new bonds, hence controlling the enthalpy of reaction (ΔH^*). The sum of these two properties at any temperature is the free energy of activation of the reaction (ΔG^*) where:

$$\Delta G^* = \Delta H^* - T \Delta S^* \qquad \text{(iv)}$$

In this regard, the MFO system is unusual in that the general form of Cytochrome P-450 has the ability to hydroxylate a wide range of geometrically unrelated xenobiotic substrates. The

number of identifiable Cytochrome P-450 isozymes is very limited in comparison with the number of known inducers. This lack of substrate specificity suggests that the MFO system has evolved a form of enthalpic control to determine the energy needed to insert activated oxygen into various substrates.

To test this concept, a three dimensional experiment was designed using the THI index to assess the relative importance of molecular shapes and bond strengths on the activity of the MFO active site (9). This experiment utilized chemicals chosen to meet specific chemical criteria of lipophilicity, structural simplicity, C-H bond strength and geometry.

Traditional experiments were designed to be two-dimensional. Animals were dosed with a chemical that "induced" the Cytochrome P-450 system. After a fixed time period to allow the animal to respond to the inducer it was killed and the prepared liver homogenate was assayed using substrates chosen for ease of analysis. Often there was no chemical similarity between the inducer and the substrate.

In our experiment, the inducers used _in vivo_ and substrates for testing _in vitro_ were chosen to be the same chemicals with C-H bond strengths that could be calculated from thermodymanic tables (10). Sprague-Dawley rats were tested with a sub-chronic 1/5 LD_{50} dose of each chemical. NADPH reduction by each microsomal preparation was measured in the presence of each chemical as a substrate under conditions for pseudo-first-order kinetics. The high background of NADPH reduction was removed by normalization with control microsomal activity. In this way, the response of the MFO system to a chemical (_in vivo_ response) and its resulting ability to metabolize other substrates (_in vitro_ response) can be independently compared to the bond strength of the chemical.

The null hypothesis (H_o, total) is that the MFO system will respond linearly to enthalpic requirement, that is, the C-H bond strength of the chosen chemicals both _in vitro_ and _in vivo_. A non-linear response would suggest an entropic or shape related response mechanism. This hypothesis will factor into two two-dimensional experiments:

$$H_o, \underline{total} = (\Sigma H_o, \underline{in\ vitro}) + (\Sigma H_o, \underline{in\ vivo}) \qquad (v)$$

(H_o, _in vitro_) is defined as the adaptation of MFO to the bond strength of the inducer such that substrates with a lower C-H bond strength would be metabolized with more efficiency _in vitro_ than the inducer as a substrate and substrates with a higher C-H bond strength with less efficiency. We have termed this null hypothesis as the _In Vitro_ self-substrate test as it examines MFO ability to handle substrates from the perspective of the inducer. There will be a H_o or self-substrate test for each

inducer in the total experiment. Subtracting the self-substrate activity from other substrates activities for an inducer reduces the activity data to a series of positive, negative and zero numbers that, when examined in relationship with the substrate bond strength will characterize the Free Energy of Activation ($\Delta G_{r-\bullet 1}$, in vitro) of the MFO system's in vitro substrate interaction.

These data are given in figure 1. Data regression analysis of seven of the self-substrate tests suggest there is a linear relationship between the bond strength of the weakest C-H bond in the substrate to be metabolized and the free energy of activation induced by the animal. Those noted by asterisks were not included in the regression as they did not conform with the overall linearity of the experiment.

The second half of the total null hypothesis, (H_o, in vivo), or the In Vivo self-substrate test examines the experiment from the perspective of the substrate. An in vitro substrate with a known weakest C-H bond strength will be metabolized more efficiently by MFO adapted to an inducer with a stronger C-H bond strength than MFO adapted to the self-substrate and metabolized less efficiently by MFO adapted to inducers with a weaker C-H bond strength. A high $\Delta G_{r-\bullet 1}$ predicts high activation adaption of MFO to a strong C-H bond.

Data regression analysis in figures 2 indicates that there are distinct relationships between C-H bond strength and $\Delta G_{r-\bullet 1}$, in vivo of the ten substrates in this experiment that can be grouped according to the chemical properties of the weakest C-H bond.

In the first category are hexamethylethane (HME), cyclohexane (CY) and dimethylbutane (DMB), which have weakest C-H bonds of aliphatic primary, secondary and tertiary character respectively. As these substrates have no functional groups, they typify the baseline or "intended" behaviour of the MFO system. Most endogenous chemicals that are controlled by cytochrome P-450 are known to be aliphatic.

In the second category are 1,1,1-trichloroethane (TCE), lindane (LIN), chloroform (CHL), which are chlorinated aliphatic compounds; cyclohexene (CE), which is an unsaturated aliphatic compounds; and 124-trichlorobenzene (124) and 135-trichlorobenzene (135), which are chlorinated aromatic compounds. All of these chemicals contain weakest C-H bonds that have been influenced by the presence of other functional groups. These substrates have a much higher $\Delta G_{r-\bullet 1}$, in vivo than expected. The two data regression lines intersect at 101 kcal, which is the C-H bond strength of a hydrogen adjacent to an aliphatic double bond.

Benzene has aromatic C-H bonds which are stronger than any in aliphatic compounds and lie at the point of intersection of

the two $\underline{in}$ $\underline{vivo}$ trends. It exhibits a much lower ΔG_{r-1}, $\underline{in}$ $\underline{vivo}$ than expected and does not fit into either linear relationship $\underline{in}$ $\underline{vitro}$. All substrate activities, especially the self-substrate rate have decreased, apparently shifting the chemical to a chemical potential of MFO adapted to a weaker C-H bond strength. Benzene has been identified as a suicidal inducer (11) and a cancer initiator, and is metabolized to an epoxide. This compound is not the intended phenol. It is water insoluble and is not eliminated from the lipid into phase II of excretion. The epoxide instead can then destroy surrounding protein by free radical decomposition, resulting in a low observed activity $\underline{in}$ $\underline{vitro}$. This apparent shift in bond strength position as a result of decreased activity in the $\underline{in}$ $\underline{vivo}$ self-substrate assay has been termed the Suicide Shift.

THE ASSESSMENT OF RISK

Defining the health risk of a chemical requires understanding of both the exposure to the chemical and the hazardous consequences of this exposure. Measurement of exposure is a problem of analytical chemistry. It requires development of convenient, reliable and sensitive methodologies that can be implemented in an extensive monitoring program. Proper measurement of exposure will identify those populations at risk.

The medical consequences or total hazard of the chemical requires an assessment and priorization of all undesirable biological changes. In the past, hazard has often been measured by the clinical presence of disease. Today, when exposure will result in the development of a disease such as cancer within several decades, the clinical definition of hazard is both imprecise and observed only after irreversible damage has occurred.

The answer to this is to develop an understanding of the interaction of the chemical with the early defense mechanisms of the body, because it is the breakdown of these subcellular systems that initiate the process of disease. As the first step in development of a science of chemical pathology, the development of a rapid screening method for chemicals would be possible. Potential problem chemicals could be identified and recommended for more detailed study. One such defense mechanism is the Mixed Function Oxidase system which is known to respond to and eliminate a wide array of chemicals. The measurable affects of chemical exposure can be observed within hours of administration. This versatility is very unusual as most enzyme systems are structure specific.

The THI index provides a mechanism to compare the total response at all levels of organization from whole body to subcellular effects of xenobiotics with control systems.

However, the most important component of this response is the adaptation of chemical potential at the active site and the behaviour of the xenobiotic at this active site. The observation of either intended or unintended behaviour here has the most potential as a predictor of chemical disease. The intended function of the MFO system is to hydroxylate hydrophobic chemicals so they can be eliminated from the body. When this behaviour occurs, the xenobiotic is removed, hence the risk of disease is eliminated.

The present experiment suggests that, as its first line of defense, the MFO system responds to the enthalpic properties of the invading chemical in both the inductive response and elimination process. This independence from the structural shape of the foreign compound is consistant with the versatility of MFO systems.

As the intricacy of living systems have been discovered, it is consistantly observed that the most conservative and efficient cellular processes have evolved. The response of the MFO system to the weakest C-H bond strength in a foreign chemical that needs to be disposed of can be recognized as a further example of this tendency. As cytochrome P-450 is a general enzyme system, all chemical structures, endogenous as well as exogenous that are present have the potential to be metabolized. The stronger the oxidation potential of the active site, the more the chemical structures are vulnerable to attack, since any bond that is weaker then the target C-H bond can be metabolized in this enthalpic system. Thus, while performing its "intended" behaviour, the MFO system must strike a balance between under response, where the chemical potential of the active site is too weak to be effective on the target compound which is also the self-substrate and over response, where essential substituents may be metabolized. Malfunction of the MFO system in either direction can lead to unintended influence on the surrounding tissue, which if not corrected could lead to the development of disease. A good example of over response and of accidental oxidation of other sites in strong C-H bond systems is Benzene (figure 2).

Benzene has a C-H bond which is stronger than any aliphatic equivalent and, as an inducer, will generate a high oxidation potential. The generated oxidation potential behaves as predicted in the In Vitro self-substrate test (figure 1). However, in the absence of an ortho/para or meta directing substituent in the ring, the aromatic C-C bond is more suseptible to attack than the target C-H bond, and unintended epoxide instead of an intended phenol will result. This will set off a subsequent chain reaction of "accidental" reactions. The epoxides are more hydrophobic than the corresponding phenol and therefore remain in the lipid and because they are chemically unstable will breakdown to give free radicals. The free radicals

propagate, destroying the surrounding protein, including cytochrome P-450. This reduces the observable substrate activity, especially for the self-substrate benzene itself. This results in a displacement on the In Vivo self-substrate test (figure 2) which is termed a suicide shift. The unpaired electrons generated by the free radicals are very soluble in lipid and very long lived, consequently they will attack lipid at double bonds. It is known that tissue high in unsaturated fats will develop cancer in the presence of radicals (12) and medically that benzene is a cancer initiator. This suggests a direct connection between the suicide shift generated by benzene and the strength of the cancer initiation capability. It is possible that the displacement of cyclohexene from the linear relationship with C-H bond strength in the In Vitro self-substrate test (figure 1) is also indicative of a suicide shift and therefore of a cancer initiator.

In the In Vivo self-substrate test (figure 2), there was also a group of chemicals containing functional groups that generated an oxidation potential greater than would be expected from the structural type of the calculated weakest C-H bond. As discussed earlier, this higher than necessary oxidation potential will increase the risk of generated free radicals and of the accidental oxidation of surrounding tissue or other xenobiotics. Although the self-substrate xenobiotics of this group will not destroy themselves as with benzene, these compounds can act as promotors of cancer by attack on less stable compounds such as unsaturated fats, generating free radicals through accidental behaviour.

In conclusion, the relationship between the MFO system and chemical C-H bond strength of the target xenobiotic provides a unique opportunity to study acute, subcellular effects of a chemical in a living system. As the analytical properties of this test is dependent upon relative response relationships, the reliability range of this assay will increase with further research. The relationship with C-H bond strength will also provide a theoretical tool for predicting biological response towards a new chemical. A chemical identified by this test as an initiator or promotor can be referred for further testing of its chemical pathology. A forth dimension of dose response can also be added to this assay. By varying the dose fed to the test animal, the critical concentration where the chemical emerges from the baseline response to behave as a promotor or initiator can be identified. This test system can also be used to identify non-toxic xenobiotics processed as intended that can be used to monitor for exposure or can be recommended for industrial or commercial uses.

REFERENCES

1. Kappas A. and A.P. Alvares. How the liver metabolizes foreign substances. Sci. American 232:22-31 (1975).

2. White, R.E. and M.J. Coon. Oxygen activation by cytochrome P-450. Ann. Rev. Biochem. 49:315-56 (1980).

3. Nebert, D.W. Multiple forms of inducable drug-metabolizing enzymes: A reasonable mechanism by which any organism can cope with adversity. Mol. Cell. Biochem. 27:27-46 (1979).

4. Conney, A.H. Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. 19:317-365 (1967).

5. Nebert, D.W. and N.M. Jensen. The Ah locus: Genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochrome P-450-mediated monooxygenases. CRC Crit. Rev. Biochem. 401-436 (1979).

6. Fouts, J.R. Factors influencing the metabolism of drugs in liver microsomes. Ann. N. Y. Acad. Sci. 104:875-880 (1963).

7. Boersma, D.C., L.J. Brownlee and B.R. Hollebone. A total induction index of metabolism for lipophilic xenobiotics. J. Appl. Toxicol. 4:107-193 (1984).

8. Hollebone, B.R. Categorization of lipophilic xenobiotics by the enthalpic structure-function response of hepatic mixed-function oxidase. Drug Metab. Rev. 17:93-143 (1986).

9. Brownlee, L.J. and B.R. Hollebone. A correlation of induced mixed function oxidase specific activity to C-H bond strengths in partially chlorinated monocyclic hydrocarbons. J. Appl. Toxicol. 6:61-66 (1986).

10. Cox, J.D. and G. Pitcher. Thermochemistry of organic and organometallic compounds. Academic Press Inc. (London) Ltd. 1970, Chapter 7.

11. Ortiz de Montellano, P.R., B.A. Mico and G.Y. Yost. Suicidal inactivation of Cytochrome P-450, formation of a heme-substrate covalent adduct. Biochem. Biophys. Res. Comm. 83:132-4 (1978).

12. French, S.W., K. Miyamoto, Y. Ohta and Y. Geoffrion. Pathogenesis of experimental alcoholic liver disease in the rat. Meth. Achiev. Exp. Pathol. 13:181-207 (1988).
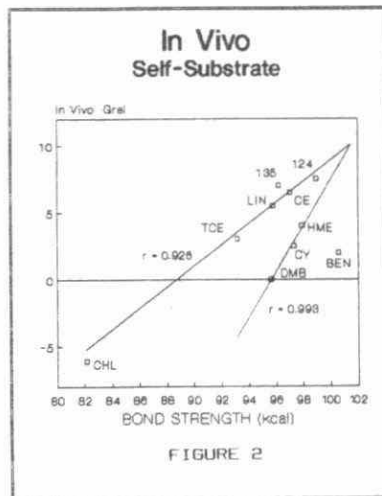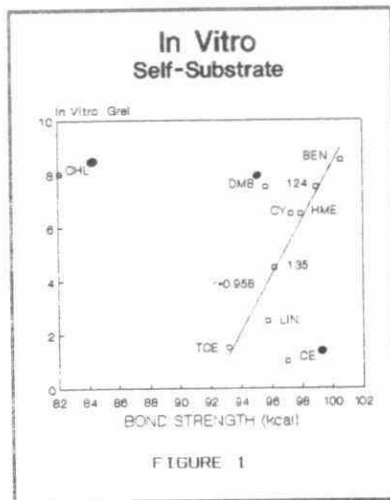
FIGURE 1

FIGURE 2

The In Vitro self-substrate test (figure 1) examines MFO ability to handle substrates from the perspective of the inducer.

The In Vivo self-substrate test examines the same data from the perspective of the substrate. Further details can be found in the text.

The substrates are: 1,1,1-Trichloroethane (TCE); Lindane (LIN); Cyclohexene (CE); Chloroform (CHL); 2,3-Dimethylbutane (DMB); Cyclohexane (CY); Hexamethylethane (HME); 1,2,4-Trichlorobenzene (124); 1,3,5-Trichlorobenzene (135) and Benzene (BEN).

D4

PROCEDURES FOR THE 2,3,7,8-SUBSTITUTED ANALYSIS
OF PCDD & PCDF AND OTHER TARGET COMPOUNDS IN
ENVIRONMENTAL SAMPLES

F. W. KARASEK[*], T. S. THOMPSON AND K. P. NAIKWADI
DEPARTMENT OF CHEMISTRY,
UNIVERSITY OF WATERLOO,
WATERLOO, ONTARIO N2L 3G1

PROCEDURES FOR THE 2,3,7,8-SUBSTITUTED ANALYSIS OF PCDD & PCDF AND OTHER TARGET COMPOUNDS IN ENVIRONMENTAL SAMPLES

Introduction:

Chlorinated pollutants have for years been a major concern because of their toxicological properties and widespread existence in the environment. Over the last decade two classes of organic compounds known as polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) have received considerable attention because of their extraordinary toxicities. These compounds have become virtually ubiquitous in the environment having been identified as contaminants in incinerator flyash, sediment, water, human milk, and biological tissue.

The basic structures of the dioxin and furan molecules are shown in figure 1 along with the eight sites which may be substituted with a chlorine atom. The spatial arrangement of one to eight chlorine atoms yields a total of 75 different PCDD isomers while the less symmmetrical furan molecule gives rise to 135 isomers. Figure 1 also shows the numbering scheme employed in the PCDD/PCDF nomenclature.

Although PCDDs and PCDFs have many similar chemical and physical properties, the toxic behaviour of individual isomrs may vary dramatically. Table I lists the LD_{50} values for several PCDD isomers [1]. The most

45

toxic dioxin and furan isomers are the 2,3,7,8-tetrachlorinated compounds, 2,3,7,8-TCDD and 2,3,7,8-TCDF respectively. There are however several other PCDD and PCDF isomers which have been found to be highly toxic. This group of toxic isomers consists of the PCDDs and PCDFs which have four to six chlorine atoms and all four lateral sites (positions 2, 3, 7, and 8 in figure 1) occupied by chlorines [2]. Thus there are twelve compounds, five PCDDs and seven PCDFs, which are highly toxic. These compounds are listed in table II.

Most PCDD and PCDF analyses have previously involved the determination of total congener levels for the tetra- through octachlorinated species and/or the isomer specific determination of 2,3,7,8-TCDD and 2,3,7,8-TCDF. The high toxicity of the ten other 2,3,7,8-substituted dioxins and furans makes it desirable to perform isomer specific determinations for these compounds.

The isomer specific determination fo PCDDs and PCDFs is hindered by the fact that no single GC column is capable of resolving all the individual isomers. Buser and Rappe prepared all of the tetra-, penta-, and hexachlorinated dioxin isomers and attempted to separate the five most toxic isomers from the remaining PCDDs [3]. Using a 55 metre Silar 10c glass capillary column, the five most toxic dioxins were reasonably well separated from the other isomers. The toxic 2,3,7,8-

TCDD was only partially resolved from 1,4,7,8-TCDD and two of the toxic hexachlorinated dioxins, 1,2,3,6,7,8-H_6CDD and 1,2,3,4,7,8-H_6CDD were found to closely elute. While there appears to have been some success in the isomer specific analysis of the highly toxic dioxins, very little work has been reported on the isomer specific determination of furans.

In this study we have investigated the combined use of HPLC and GC separation techniques for the isomer specific determination of 2,3,7,8-substituted dioxins and furans.

Experimental:

Individual PCDD and PCDF standards were obtained from the Ontario Ministry of the Environment. All organic solvents were distilled in glass by the supplier (BDH Chemicals Inc., Toronto, Ontario) and were suitable for pesticide analysis. A flyash extract used to investigate the separation capabilities of the alumina normal phase HPLC (NP-HPLC) fractionation. This extract was obtained by Soxhlet extracting approximately 40 grams of municipal waste incinerator flyash (Commissioner Street, Toronto, Ontario) with 350 mL of benzene for 24 hours. The extract was fractionated on silica NP-HPLC to isolate the dioxins and furans from the bulk of the matrix prior to performing the alumina NP-HPLC fractionation.

47

HPLC fractionations were accomplished using a Waters liquid chromatographic system which consisted of three model 510 pumps and a model 481 variable wavelength ultraviolet (UV) detector. The HPLC is controlled by a Waters 820 chromatography workstation consisting of an NEC APCIV personal computer and monochrome monitor. The workstation is linked to the pumps and detector by a system interface module.

Sample was introduced into the HPLC via a loop injector which consisted of a Rheodyne six-port valve with a 20 microlitre sample loop. The organic solutions are loaded into the loop through a needle port using a flat-tipped 100 microlitre Hamilton syringe.

The sample fractionations were achieved using an analytical scale alumina column (25 cm x 0.4 cm i.d.). This column was prepared in our laboratory using a Shandon packing pump (Shandon Southern Instruments Inc., Wewickley, Pennsylvania). A slurry of 5 micron alumina particulates (Rayonics Scientific Inc., Downsview, Ontario) in methanol was packed under a pressure of approximately 9000 psi.

The gradient elution program used for the separation is given in table III.

The HPLC effluent was collected using a Gilson model 201 fraction collector. The fraction collector was set up to collect the effluent at discreet intervals as established through the injection of standard

solutions of PCDDs and PCDFs under the identical gradient elution program. The fraction collection times are listed in table III.

All gas chromatography-mass spectrometry analyses were performed using a Hewlett-Packard HP5987A GC-MS. The HP5880A gas chromatograph is linked to the quadrupole mass spectrometer by a direct capillary interface. This permits the end of the GC column to butt up against the mass spectrometer ion source. All of the GC effluent therefore enters the mass spectrometer. A cool on-column injector and 30 metre DB-5 fused silica capillary column (0.32 mm i.d.) were used for all GC-MS analyses. The GC-MS is controlled by an HP1000 data system which is also linked to various peripheral devices. For the selected ion monitoring determination of PCDDs and PCDFs, three ions were chosen for each congener group. These ions corresponded to the most intense peaks in the molecular ion cluster for the various congeners.

Results and Discussion:

Two standard solutions containing a total of 15 PCDD and PCDF isomers were injected on alumina NP-HPLC. The resulting chromatograms are shown in figure 2. By collecting fractions at the intervals listed in table III and subsequently analyzing by GC-MS, the elution behaviour of the dioxins and furans was determined.

49

Clearly the elution behaviour does not depend solely upon the degree of chlorination and therefore it may be possible to exploit this selectivity. To investigate this possibility, a flyash extract believed to contain virtually all PCDD and PCDF isomers was fractionated on alumina NP-HPLC.

The flyash extract, which had been pre-cleaned using silica NP-HPLC, was injected on alumina NP-HPLC and fractionated using the conditions described in table III. The HPLC UV trace for the flyash extract along with the fraction collection intervals is shown in figure 3. Each of the collected fractions was subsequently concentrated to 20 microlitres and analyzed by GC-MS. Figures 4 through 10 show the reconstructed ion chromatograms for the dioxins and furans in the fractions as determined by selected ion monitoring. The highly toxic isomers have been labelled in their respective chromatograms.

As expected, 2,3,7,8-TCDF is collected in the fourth fraction. More importantly, the majority of the other TCDF isomers elute within the first three fractions. A single TCDF isomer elutes after 2,3,7,8-TCDF in the fifth fraction. The alumina NP-HPLC separation appears to be very effective in isolating the toxic 2,3,7,8-TCDF from the remaining TCDF isomers.

The majority of the total TCDD elutes within the first two fractions. 2,3,7,8-TCDD elutes between 16 and

18 minutes along with 1,2,3,4-TCDD and three other TCDD isomers. The 1,2,3,4-TCDD and 2,3,7,8-TCDD are not resolved by the DB-5 column however many other columns will resolve these two isomers.

Fraction 3 also contains several other highly toxic isomers as indicated in figure 6. Two of the toxic P_5CDFs, $1,2,3,7,8-P_5CDF$ and $2,3,4,7,8-P_CDF$, are separated from the bulk of the remaining isomers which elute largely in fractions 1 and 2.

Conclusions:

The results obtained for the flyash extract indicate that the alumina column exhibits considerable selectivity. Many of the toxic isomers can be separated using a combination of HPLC and GC techniques. A further experiment involving fish tissue samples fortified with labelled dioxins and furans is currently underway and should yield more information regarding the applicability of the method to environmental samples.

References:

1.   G. Choudhary in "Chlorinated Dioxins and Dibenzofurans in the Total Environment", G. Choudhary, L.H. Keith, and C. Rappe (ed.'s), Butterworth Publishers, Boston, Massachusetts, 1983, 416 pp.

2. H.R. Buser and C. Rappe; Anal. Chem., 56(3), 442-448, 1984.

3. H. R. Buser and C. Rappe; Anal. Chem., 52(14), 2257-2262, 1980.

TABLE I

Lethal Dosages of Selected Dioxins

| DIOXIN ISOMER | LETHAL DOSAGE (LD_{50} ug/kg)* | |
	GUINEA PIGS	MICE
$2,7-D_2CDD$	> 2,000,000	> 8,000,000
$2,8-D_2CDD$	> 300,000	> 150,000
$2,3,7-T_3CDD$	29,444	> 3,000
$2,3,7,8-T_4CDD$	2	284
$1,2,3,4-T_4CDD$	> 1,000,000	
$1,2,3,7,8-P_5CDD$	3	338
$1,2,4,7,8-P_5CDD$	1,125	> 5,000
$1,2,3,6,7,8-H_6CDD$	70-100	1,250
$1,2,3,7,8,9-H_6CDD$	60-100	1,440
$1,2,3,4,6,7,8-H_7CDD$	180	

* taken from reference 1

TABLE II

Highly Toxic PCDDs and PCDFs

PCDDs:

2,3,7,8-TCDD

1,2,3,7,8-P_5CDD

1,2,3,6,7,8-H_6CDD

1,2,3,7,8,9-H_6CDD

1,2,3,4,7,8-H_6CDD

PCDFs:

2,3,7,8-TCDF

1,2,3,7,8-P_5CDF

2,3,4,7,8-P_5CDF

1,2,3,4,7,8-H_6CDF

1,2,3,6,7,8-H_6CDF

1,2,3,7,8,9-H_6CDF

2,3,4,6,7,8-H_6CDF

TABLE III

Alumina Normal Phase HPLC
Gradient Elution Program

TIME (min)	FLOW RATE (mL/min)	MOBILE PHASE COMPOSITION	
		% HEXANE	% DICHLORO-METHANE
0.0	2.0	100.0	0.0
10.0	2.0	100.0	0.0
11.0	2.0	95.0	5.0
30.0	2.0	95.0	5.0
35.0	2.0	0.0	100.0
55.0	2.0	0.0	100.0
60.0	2.0	100.0	0.0

Fraction Collection Times

FRACTION #	COLLECTION TIME
1	12 - 14
2	14 - 16
3	16 - 18
4	18 - 20
5	20 - 24
6	24 - 26
7	26 - 30

55
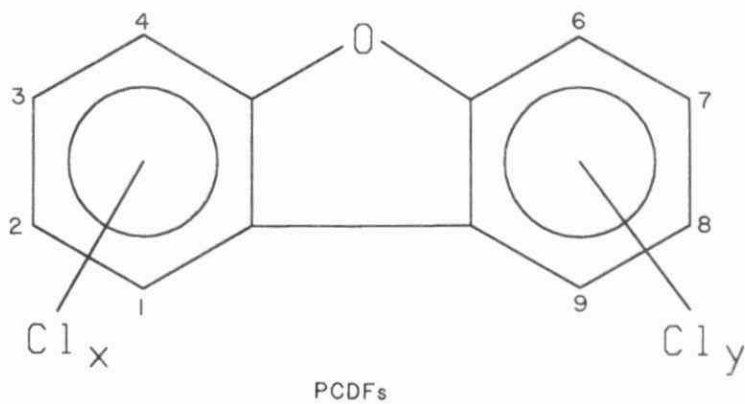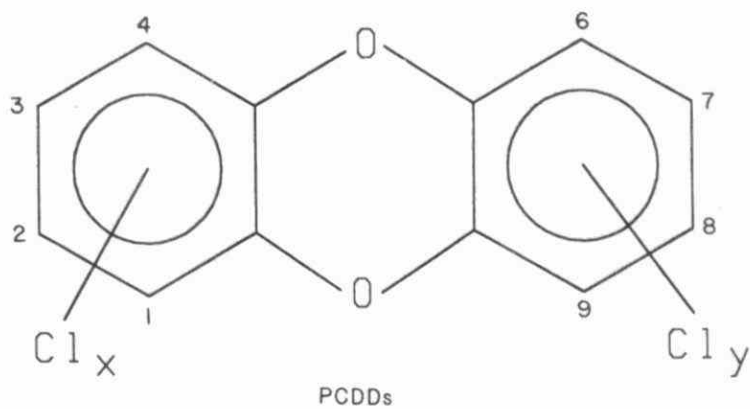
PCDDs

PCDFs

FIGURE 1: Structure of PCDDs and PCDFs

56
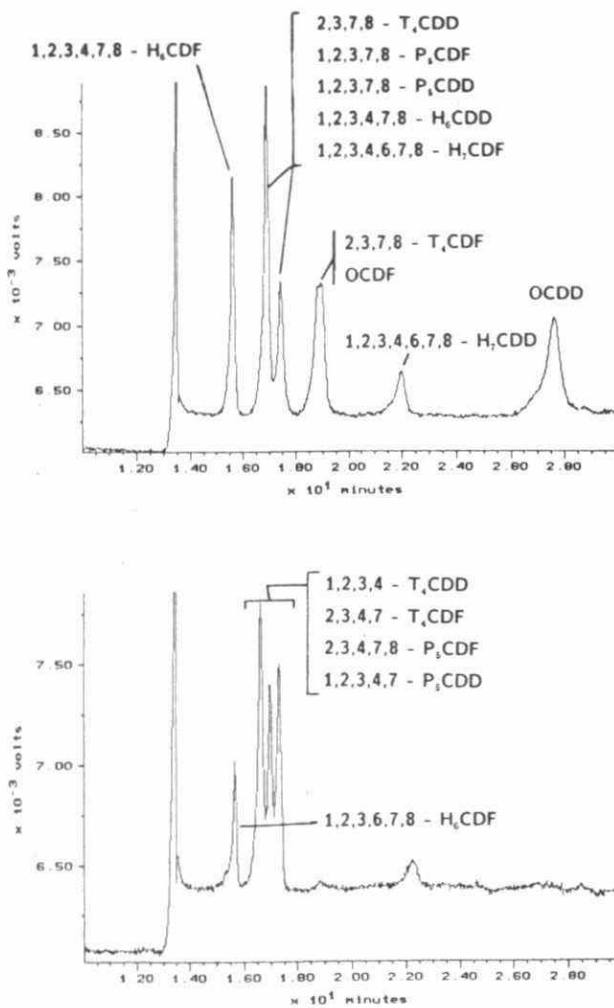
FIGURE 2: Alumina NP-HPLC of PCDDs and PCDFs

57
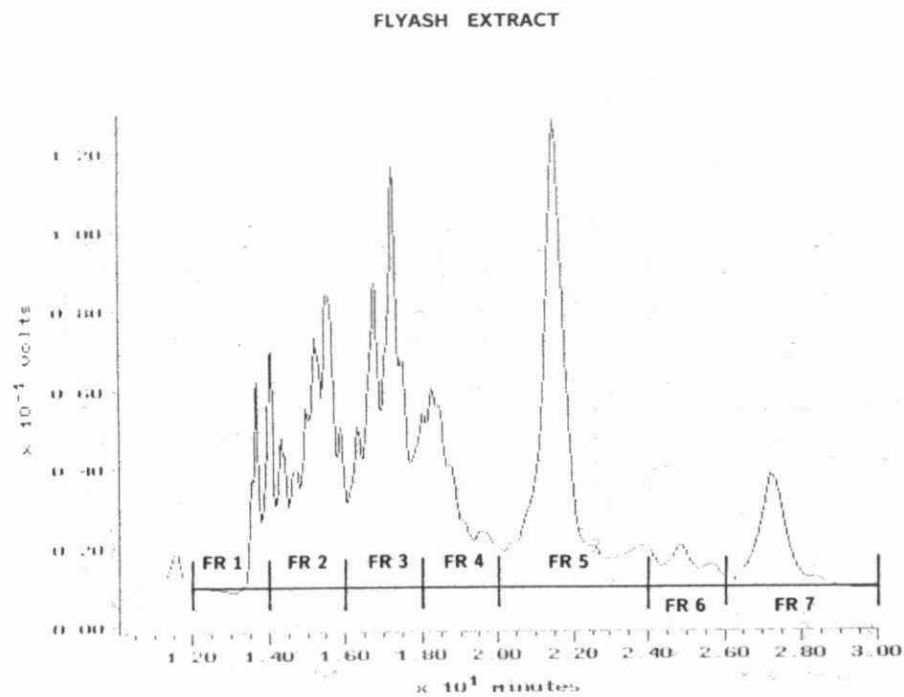
FLYASH EXTRACT

FIGURE 3: NP-HPLC fractionation of flyash extract.
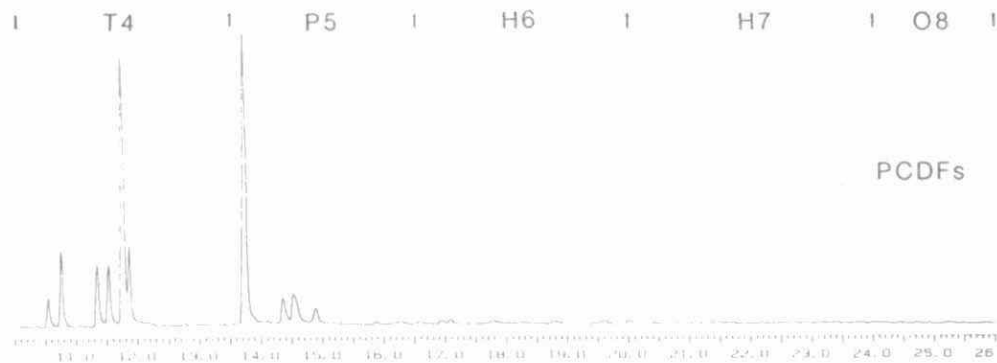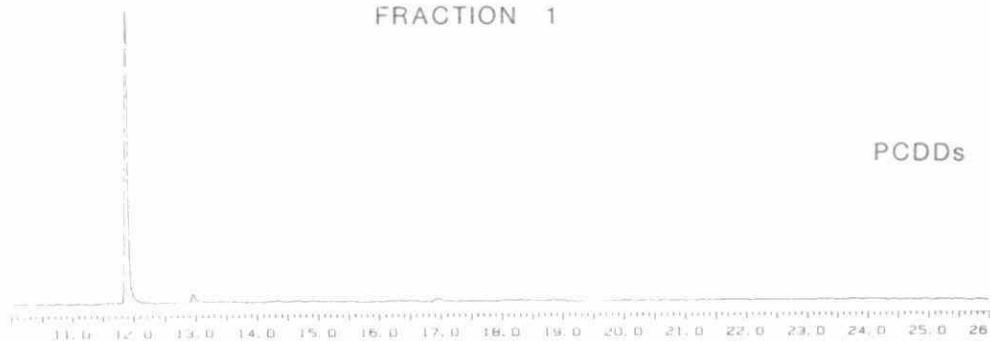
FIGURE 4: RIC of flyash fraction #1.
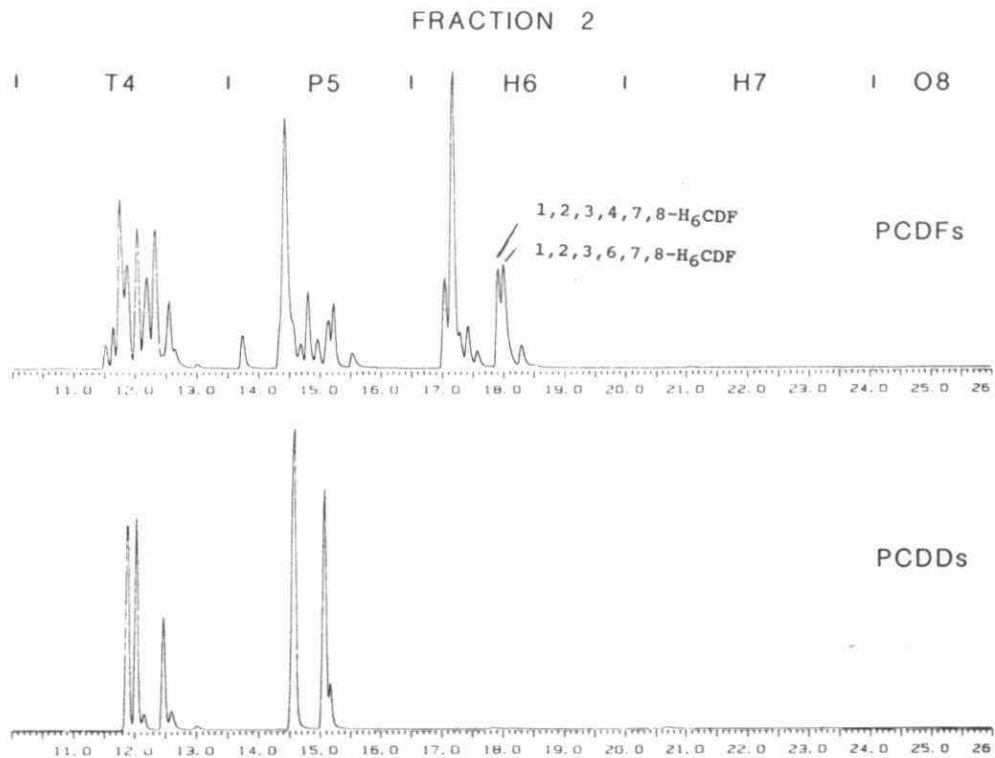
FIGURE 5: RIC of flyash fraction #2.
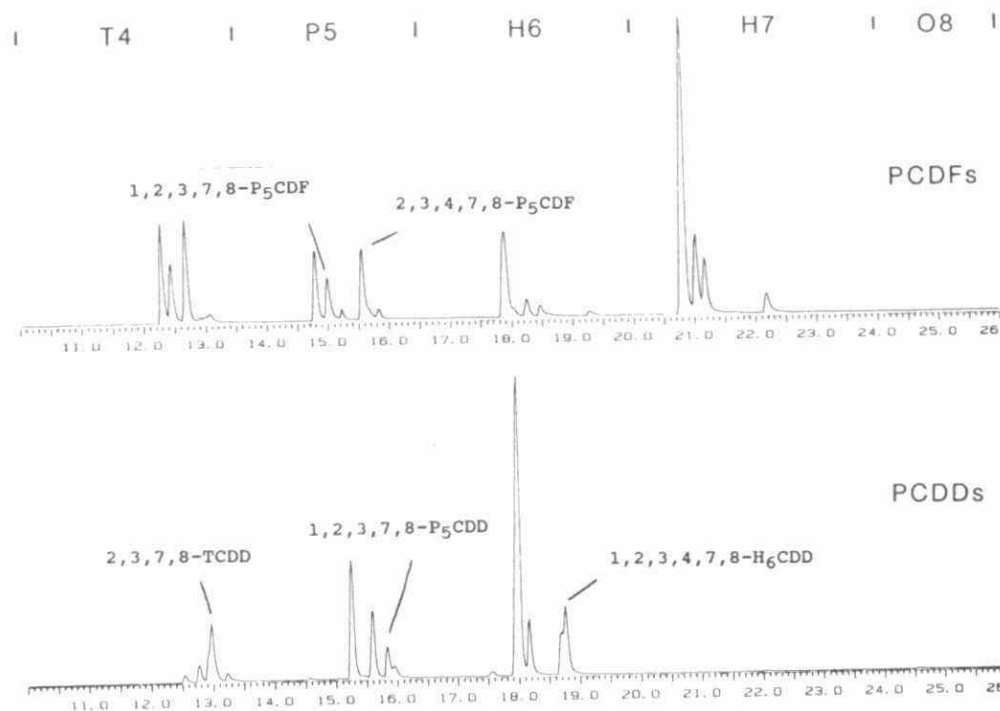
FIGURE 6: RIC of flyash fraction #3.
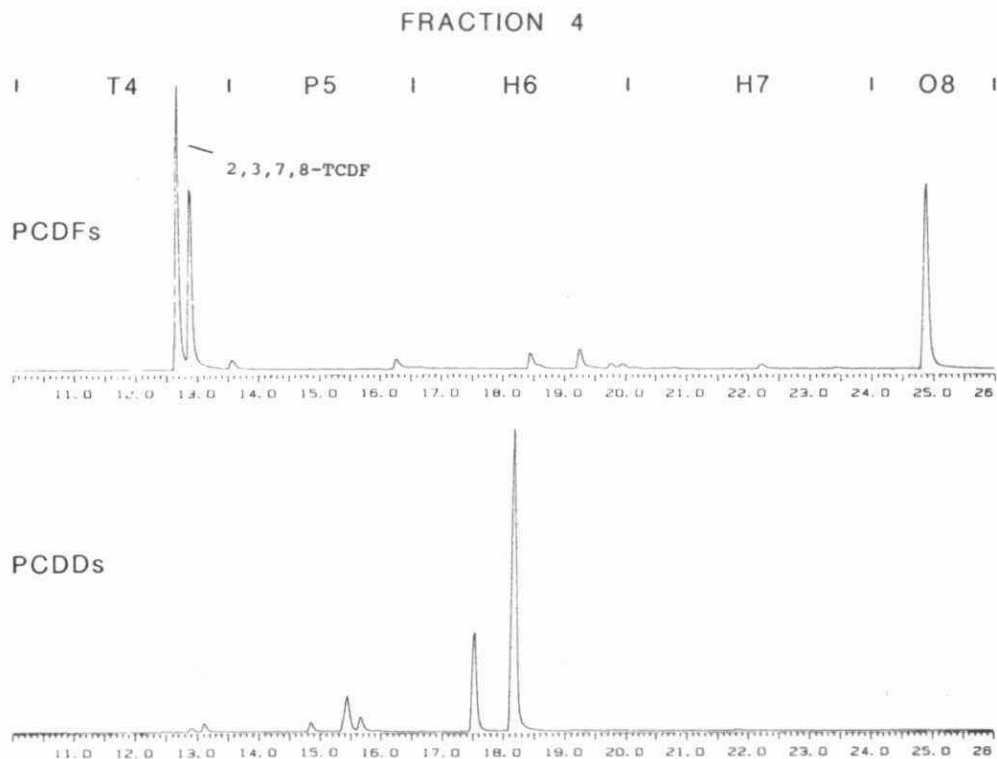
FRACTION 4

FIGURE 7: RIC of flyash fraction #4.
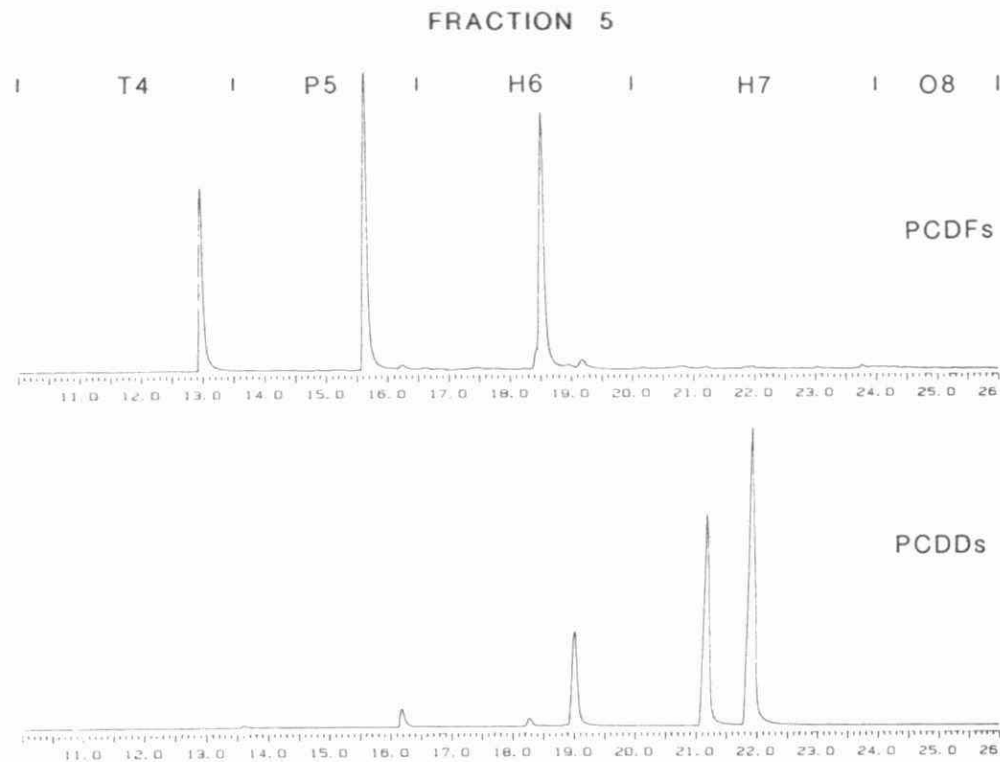
FIGURE 8: RIC of flyash fraction #5.

FRACTION 6

| T4 | P5 | H6 | H7 | O8 |

PCDFs


```
 11.0  12.0  13.0  14.0  15.0  16.0  17.0  18.0  19.0  20.0  21.0  22.0  23.0  24.0  25.0  26
```

PCDDs


```
 11.0  12.0  13.0  14.0  15.0  16.0  17.0  18.0  19.0  20.0  21.0  22.0  23.0  24.0  25.0  26
```

FIGURE 9: RIC of flyash fraction #6.
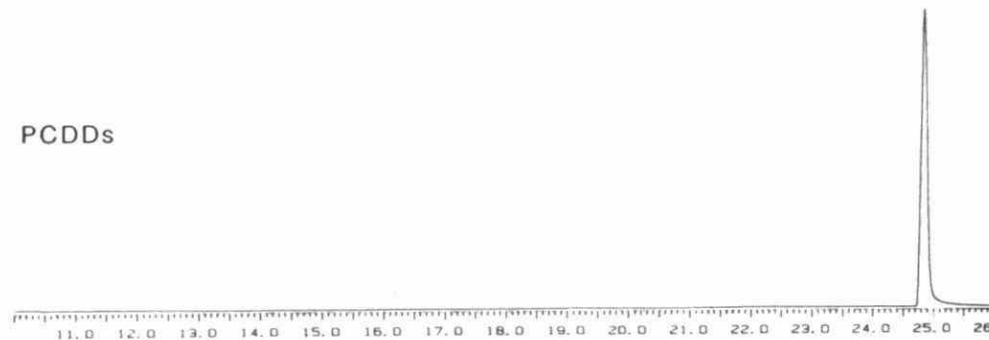
FIGURE 10: RIC of flyash fraction #7.

D5

THE CLOSED-LOOP STRIPPING TECHNIQUE APPLIED TO POTABLE WATER
TO SOLVE TASTE AND ODOUR PROBLEMS.

J-P. Palmentier[*], D. Robinson and V.Y. Taguchi.
Ontario Ministry of the Environment, Laboratory Services Branch,
P.O. Box 213, Rexdale, Ontario M9W 5L1

The occurrence of objectionable taste and odour problems in
potable water is mainly a seasonal problem when not associated
with industrial effluent discharges. Microbiological activity has
been found to be responsible for causing earthy-musty odours in
water especially in the spring and fall seasons. Two
compounds identified as metabolites from microbiological activity
are geosmin and 2-methylisoborneol [1,2]. Other compounds causing
taste and odour problems are 2-isopropyl-3-methoxypyrazine, 2-
isobutyl-3-methoxypyrazine and 2,3,6-trichloroanisole [3,4,5].
These compounds have threshold odour concentrations in the low
nanogram per litre levels. Analysis at these low levels requires
an alternative method to the conventional purge-and-trap
technique. Such an alternative is the closed loop stripper.

Closed loop stripping (CLS) was developed by Grob in 1973 for the
analysis of organic substances in potable water [6]. This
technique involves recirculation of a headspace gas from 1L of
water in a closed system for 2 hours. Components are stripped
into the recirculating gas and adsorbed onto an activated
charcoal trap. Compounds are extracted from the trap using a
small amount (15uL) of solvent such as carbon disulfide or
methylene chloride. An aliquot of the extract is analyzed using

high resolution gas chromatography-low resolution mass spectrometry.

Conventional CLS is limited by the fact that only a fraction of the extract is analyzed. To decrease the detection limits for geosmin and methylisoborneol, a study involving collection of the stripped components onto Tenax GC or TA or layered traps with subsequent thermal desorption of the trap directly into a GC-MS is in progress. Results from these studies will be presented.

References:

1. Gerber, N.N.,and Lechevalier, H.A., Appl. Microbiol. 19 (1965) 935.

2. Rosen, A.A, Mashni, C.I., Safferman, R.S., Water Treat. Exam. 19 (2) (1970) 106.

3. Gerber, N.N., J. Chem. Ecol. 3 (1977) 475.

4. Buttery, R.G., Seifert, R.M., Gauadagni and Ling, L.C., J. Agric. Food Chem. 17 (1969) 1322

5. Reinhard, M., and McCarty, P.C.,"Characterization of Organic Materials at Water Factory 21" in Chemistry in Water Reuse, Vol. 2, W.J. Cooper , ed., Ann Arbor, MI., Ann Arbor Science Publishers Inc, 1981.

6. J. Chromatogr. 84 (1973) 255.

D6

SOLID SUPPORTED REACTIONS IN ENVIRONMENTAL ANALYSES:
Rosenfeld J.M.[1] and Matthew-Ahlang F.[2]

1. Department of Pathology, 2. Central Analytical
Laboratory, Faculty of Health Sciences, McMaster University;
1200 Main St. W. Hamilton Ont. L8N 3Z5

INTRODUCTION:

Solid phase processes are an approach to simplifying and
automating analytical procedures for the determination of organic
compounds from aqueous matrix. The most common technique is that
of adsorption/desorption on reverse phase column chromatography
which serves to isolate and concentrate organics from aqueous
matrix. Isolation and concentration, however, are frequently
insufficient to achieve the sensitivities for environmental
analysis and derivatization is frequently required. As a rule
such analytical reactions are carried out in solution and off-
line which complicates sample handling and makes automation more
complex. Solid supported reaction were investigated to deal with
problems of solution derivatization and of-line reactions (1-5).
This work was based on the hypothesis that analytical methods
using complete solid phase sample preparation technology will
lead to a more facile development of automated procedures.

Determination of the chlorophenoxy acetic acid herbicides
and their phenolic breakdown products usually involves
derivatization of the acidic functionality. Methylation or
pentafluorobenzylation are the usual reactions used to prepare
the derivatives . We investigated pentafluorobenzylation of
these analytes on a solid support of a XAD-2 a macroreticular
styrene/divinylbenzene cross linked copylmeric resin. In
conjunction with this derivatization procedure we also developed
in-line chromatographic separation of derivatized analytes from
each other and from derivatives of interferences.

EXPERIMENTAL:

Apparatus: The pentafluorobenzyl (PFB) derivatives of pure
analytes were determined on a Helwett-Packard (H-P) 5790 GC
equipped with a pulse linearized ECD and a J & W fused capillary
column DB-1, 30 M X 0.321 MM with film thickness 0.25 μM. The
output of the detector was monitored on a H-P 3390A recording
integrator. Hydrogen was used as a carrier gas with linear
velocity of 62 cm/sec at 180°C and 10 % methane in argon was used
as a make-up gas at a flow rate 15 ml/min.

Reagents: Pentafluorobenzyl bromide (PFBBr) was purchased
from Caledon Laboratories, Georgetown, Ontario. The
macroreticular resin, XAD-2, a cross-linked copolymer of
styrene/divinylbenzene was obtained from BDH Laboratories,
Toronto, Ontario and was cleaned and stored as previously

described (2,3). Florisil and basic alumina were purchased from
Supelco (Canada) Oakville Ont. Disposable 1 ml Supelclean
columns used for packing the Florisil and basic Alumina semi-
preparative column and a vacuum module were also purchased from
Supelco (Canada) Oakville Ont. Solvents were purchased from the
usual commercial suppliers, such as Fisher, BDH and Aldrich
Canada. Pure analytes were obtained from the E.P.A Repository.

All the glass-ware was silylated by standard procedures.
Glass-ware and plastic-ware was washed with methylene dichloride,
methanol, acetonitrile and dried prior to use.

Preparation of PFB Derivatives: Pentafluorobenzyl
derivatives of the pure analytes were prepared by stirring of the
organic acid in acetone with PFBBr with K_2CO_3 as the base.
Reaction work-up consisted of evaporating the acetone, extracting
the residue with CH_2Cl_2 and finally washing the organic phase
with distilled water. The PFB products were purified by thin-
layer chromatography (3).

Derivatization and Isolation: Two hundred mg of XAD-2 was
added to a 16X100 mm screw cap vial and wetted with 100 uL of
acetonitrile. Four mL 0.1 M Phosphate buffer at pH 7.4
containing analyte was added to these vials followed by 100 uL of
PFBBr in hexane (1/9 v/v). The reaction mixture was shaken for 2
hours in a water bath at 40°C. The resin was isolated by
filtration in a 5 mL Supelclean cartridge and washed with
distilled water. After the interstitial water, was removed by
suction 100 µL of acidified 2,2 dimethoxypropane was added and
allowed to remain in contact with the resin for 15 minutes.
Excess scavenging reagent and products of hydrolysis (acetone and
methanol) were removed with a gentle stream of nitrogen at room
temperature. The cartridge was then inserted linked in series to
a 1 mL cartridge containing Florisil and a 1 mL cartridge
containing basic alumina. Derivatized phenols were eluted from
the link with hexane/toluene (99/2 v/v). The Florisil and
Alumina columns were split and PFB-2,4 dichlorophenoxy acetate
was eluted with hexane/acetone (99/1 v/v)

Gas Chromatography: Samples were analyzed by gas chromatography
with electron capture using the column described above and the
following following temperature conditions: 180-215°C at
4°C/min; 215°C to 300°C at 20 °C with a 2 minute hold at 300°C.

Results and Discussion:

In the herbicide problem there are several distinct issues
that need to be dealt with in the development analytical
techniques based on pentafluorobenzylation using solid supported
reactions. Firstly there are two distinct groups of analyte that
are involved: the carboxylic acids and the corresponding phenols
that are the environmental breakdown products. Derivatization

our hands, the pentafluorobenzyl (PFB) ester of the acid was not recovered at alkaline conditions whereas PFB ethers of 2,4-dichlorophenol, 2,4,5-trichlorophenol and pentachlorophenol were formed and isolated (Table I). The reason for this is not clear but it was possible that under alkaline conditions here may have been hydrolysis of 2,4 dichlorophenoxy acetic acid to 2,4-dichlorophenol. However, a PFB ether of the corresponding phenol was not recovered from XAD-2 supported pentafluorobenzylation of 2,4 dichlorophenoxy acetic acid at alkaline pH. The problem was not investigated further at that point, as we found that simultaneous derivatization of the analytes and recovery of PFB derivatives of all the analytes was possible if derivatization was carried out at pH 7.4. These were the only phenols that, in our experience, were derivatized at pH 7.4 and probably reflected the lowered pKa of this class of phenol.

Derivatization of analytes was necessary but insufficient for developing methods of high sensitivity. The organic acid matrix of lipophilic compounds that can interfere, even when limited to ordinary laboratory reagents and water, is quite complex. Separation of derivatized analyte from interferences was an essential consideration.

A primary requirement of any separation technique is effective transfer of analyte to the chromatographic phase. In the case of solid supported reaction such transfer involved two steps: elution from the resin in high yield followed by transfer to the normal phase without undue spreading of the derivative over the length of the column. This combination of requirements dictated the elution from the resin in the most lipophilic solvent possible and this required development of effective drying conditions.

After derivatization the reaction products remain adsorbed on a surface that is in turn coated with water. This makes elution with lipophilic solvents inefficient (Fig. 1 a) by inhibiting contact of eluant with the surface of the resin. As a result 50-70% is eluted in hexane and the remainder requires elution with more polar solvents. If the latter group of solvents were used to elute from the resin onto the normal phase then, not surprisingly, there was very no effective separation of derivatized analyte from interferences.

The first step of the separation problem was thus reduced to the drying of the resin. This is a standard requirement following adsorption of analyte from water using a reverse phase columns composed of alkylsilica or XAD-2. This is usually affected by heating or with vacuum in conjunction with a stream of nitrogen. This approach, however, cannot be used when dealing with the low molecular weight, and hence volatile, analytes and derivatives under investigation. Accordingly we tested a volatile water scavenger 2.2-dimethoxypropane to dry the resin

(Fig. 2). This proved to be effective after treatment of the resin and after evaporation at low temperature and at atmospheric pressure of 2,2-dimethoxypropane and it's hydrolysis products it was possible to elute all the analytes from the resin in hexane.

$$CH_3-\underset{\underset{\displaystyle CH_3}{\overset{\displaystyle O}{|}}}{\overset{\overset{\displaystyle CH_3}{|}}{\underset{|}{\overset{|}{C}}}}-CH_3 \quad + \quad H_2O \quad \xrightarrow{\quad H^+ \quad} \quad 2\ CH_3\,OH \quad + \quad CH_3-\overset{\overset{\displaystyle O}{\|}}{C}-CH_3$$

Figure 2.   Reaction of 2,2 dimethoxypropane with water.

Florisil was not effective at separating the PFB derivatives of the phenolic analytes from the interferences since both these products eluted with hexane (Fig. 3). As a result a linked system using basic alumina as the normal phase was investigated. This phase allowed isolation of the PFB ethers in a reasonably clean isolate (Fig 4) 5 ng/mL. A difficulty was encountered with PFB-2,4 dichlorophenoxy acetate which could not be recovered from this normal phase (Table I). Thus the unexpected behaviour of 2,4 dichlorophenoxy acetic acid and it's PFB derivative necessitated rther modification of the linked system.

It was proposed that clean-up of the PFB ethers and PFB ester could be affected by a linked system which allowed lipophilic eluate from the Florisil to be transferred directly to a column of basic Alumina thus trapping the interferences and allowing the PFB ethers to elute. The link between the two normal phase columns was then broken and the PFB-2,4 dichlorophenoxy acetate was eluted from the Florisil with acetone in hexane (Fig 5).
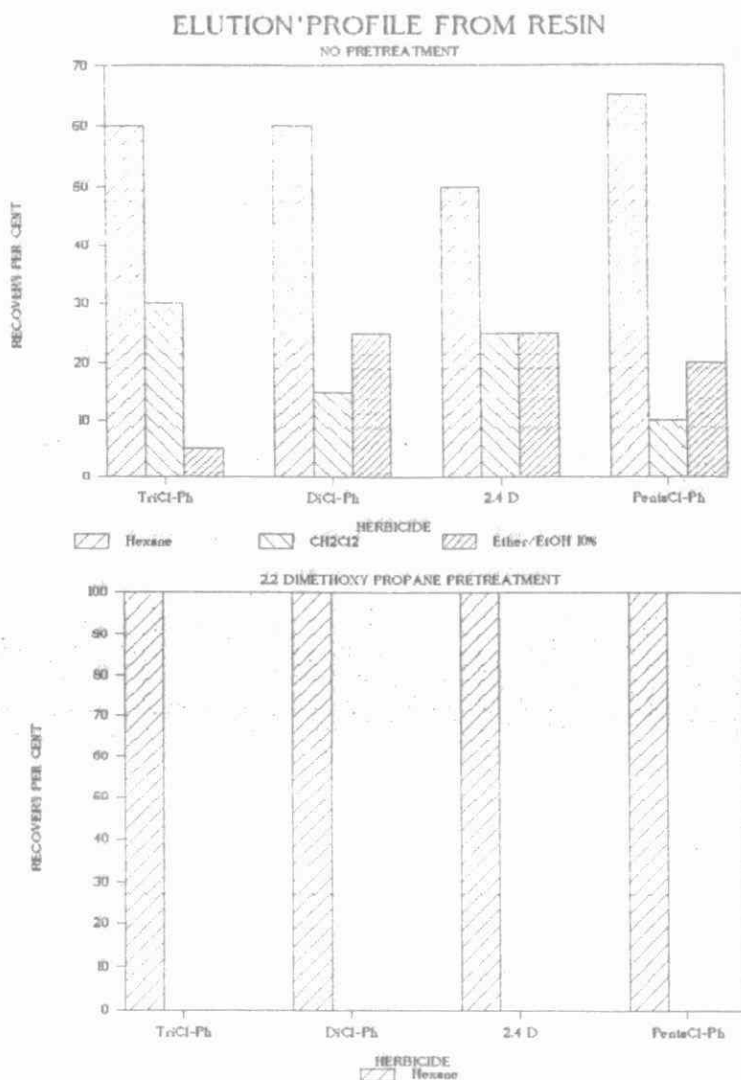
Figure 1. Elution profile of derivatized analytes from resin with
A) no treatment; B) drying with 2,2-dimethoxypropane.
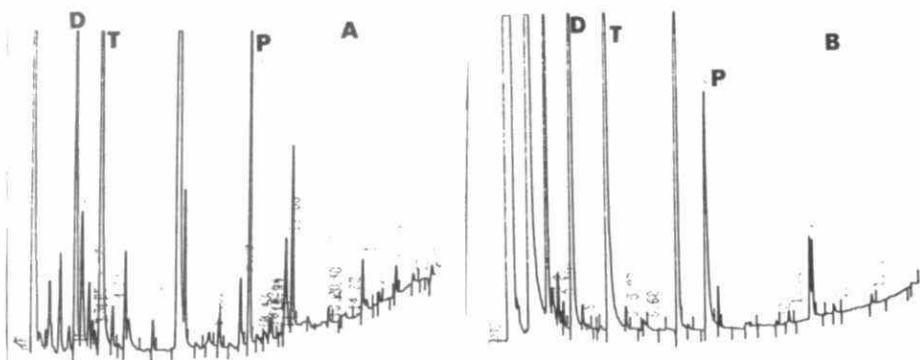
Figure 3. Clean-up of reaction for derivatization of phenols on XAD-2: A) Hexane eluate from Florisil; B) Hexane/ Toluene (99/1 v/v) eluate from Florisil/Alumina link. (For analysis of B tempreature program was started at 170°C to compensate for interferences in the solvent front). D = 2,4-dichlorophenol; T = 2,4,5-tricholorophenol and P = pentachlorophenol
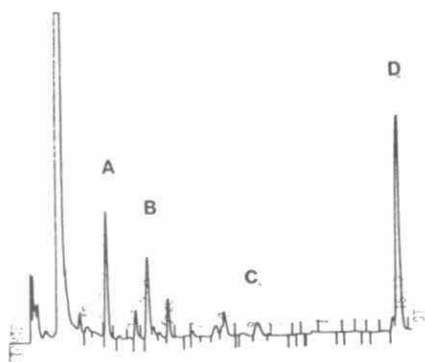
Figure 4. Determination of 5 ng/mL of polychlorinated phenols from water: PFB derivatives eluted in the Hexane/Toluene (99/1 v/v) eluate from Alumina.
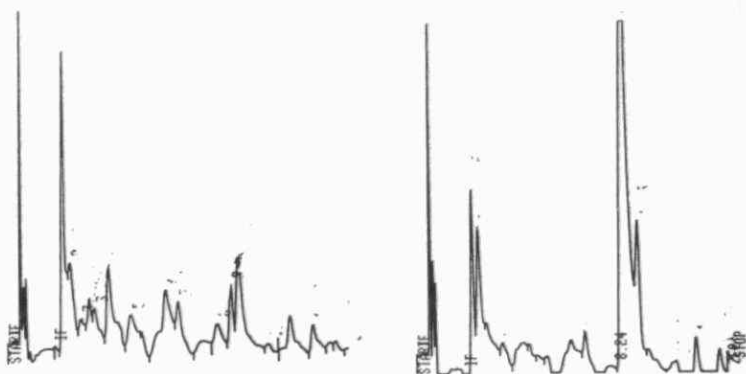
Figure 5. A) Hexane/Acetone (95/5 v/v) eluate of blank B) Hexane/Acetone (95/5 v/v) eluate of sample containing 2,4 Dichlorophenoxy acetic acid (50 ng/mL).

REFERENCES:

1. Rosenfeld, J.M.; Walker, C.H.; Brown, G.M.; Sprung, C.H., J. Chromatogr. 1985, 325, 309.

2. Rosenfeld, J.M.; McLeod, R.; Foltz, R.L. Anal. Chem. 1986, 58, 716.

3. Rosenfeld, J. M.; Mureika-Russell, M.; Yeroushalmi, S. J. Chromatogr. 1986, 358, 137.

4. Rosenfeld, J.M.; Orvidas, M.C.; Hammerburg, O.; J. Chromatogr. 1986, 378, 9.

5. Rosenfeld, J.M.; Osei-Twum, E; Yeroushalmi, S.; Anal. Chem. 1986, 58, 3343.

77

SYNTHESIS AND USE OF LIQUID CRYSTALLINE POLYSILOXANE SUBSTRATE IN CAPILLARY COLUMN GC-MS FOR ISOMER SPECIFIC SEPARATION OF TOXIC ISOMERS OF PCDD AND PCDF.

K. P. Naikwadi* and F. W. Karasek,

Department of Chemistry, University of Waterloo, Waterloo, Ontario, N2L 3G1.

ABSTRACT:

The synthesis and characterization of a variety of mesomorphic (liquid crystalline) side chain polysiloxane substrate known to be useful as gas chromatographic stationary phases , are described and discussed. The synthetic scheme is based upon the hydrosilation reaction that occurs when precursor liquid crystalline alkene compounds are contacted with polymethylhydrosilane in the presence of a platinum catalyst. Liquid crystalline polysiloxane offer unique selectivity when used as stationary phase in capillary gas chromatography. Separation on such column occurred based on the size and shape of the solute molecules. Separation and quantitation of the most toxic isomers of polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) generally requires the use of long polar capillary columns, that are inadequate for analysis of total PCDD, PCDF. However, the liquid crystalline polysiloxane capillary column shows unique selectivity for separation of 2,3,7,8-TCDD and TCDF, and analysis of total PCDD and PCDF in same GC-MS run can be carried out. The advantages of the liquid crystalline polysiloxane capillary column over conventional capillary columns used in analysis of PCDD and PCDF will be discussed.

Introduction:

Isomer-specific separation of polycyclic aromatic hydrocarbons (1), polychlorinated biphenyls (PCBs) and insect sex pheromones (2,3) have been reported using low molecular weight liquid crystalline stationary phases in gas chromatography (GC). Most recently , liquid crystalline polymers (LCPs) have been developed to use as stationary phases in GC and supercritical chromatography (SFC)(4-9). There are several advantages of LCP stationary phases over low molecular weight liquid crystals. The distinct advantage of thermal stability and uniform thin film formation brought in by their polymeric nature has provided the high efficiency sorely lacking in the monomeric liquid crystals.

There are two types of LCPs, main chain LCPs, that contains liquid crystalline rigid core connected by flexible spacers, and side chain LCPs, containing liquid crystalline moieties connected to the main chain as pendent groups. So far, only side chain LCPs have been used in GC and SFC. Synthesis and use of a series of liquid crystal polyacrylates in GC and SFC for isomer specific separation has been reported (4-7). Most recently developed liquid crystalline polysiloxanes (LCPSs) have shown great promises for separation of polycyclic aromatic hydrocarbons (10-12), sulphur compounds (13), and PCDD/PCDF (14). Thermal stability and efficiency of LCPS columns has been reported to be comparable to that of conventional capillary columns. In addition, LCPSs show high isomer specific selectivity for various classes of environmental pollutants.

In this paper synthesis of a series of LCPSs has been reported. A method to develop efficient capillary columns using LCPSs is described. The results of separation of environmental samples and standards on a conventional column (DB-5) and on a column developed in this study are demonstrated.

80

Experimental:

All chemicals were purchased from Aldrich. The PCDD standard was synthesised in our laboratory. Fly ash sample extract was obtained by soxhlet extraction of Ontario fly ash. The chemical structures of all new monomers and polysiloxanes synthesised were determined by NMR spectral analysis. General reaction scheme is shown in Figure 1.

LIQUID CRYSTALLINE POLYSILOXANE
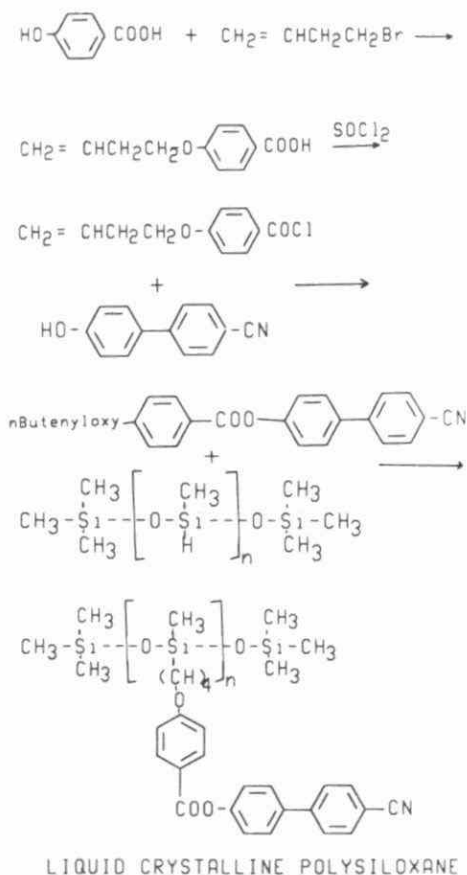
FIGURE 1. REACTION SCHEME FOR SYNTHESIS OF LCPSs

Synthesis of Liquid Crystalline Polysiloxanes:

A representative procedure for synthesis of butenyloxy benzoic acid , its chloride, alkene monomer and polysiloxane are describe below.

4-Butenyloxy benzoic acid:

Potassium hydroxide 12.32 g (0.22 mol) was dissolved in ethanol water mixture (300:100). 4-Hydroxy benzoic acid (13.8 g, 0.1 mol) was added to potassium hydroxide solution. Potassium iodide (0.1 g) was added and mixture was heated to reflux temperature. 4-Bromo-1-butene (14.8 g, 0.11 mol) was added and the mixture was refluxed overnight. Ethanol (150 mL) was removed by distillation and the residual reaction mixture was cooled and then acidified using concentrated hydrochloric acid. The solid carboxylic acid was removed by filtration and washed with water. The air dried crude acid was recrystallised from ethanol to give 10.2 g of plates.

General procedure for esterification to form monomeric liquid crystals:

An alkenyloxy carboxylic acid was reacted at room temperature with an excess thionyl chloride containing a drop of dimethyl formamide to obtained a clear solution. The mixture was then heated to 50 ^{0}C for 2 h. and excess thionyl chloride removed by vacuum distillation. The acid chloride was dissolved in 15 mL of dry pyridine and equimolar p-substituted hydroxy benzene or biphenylene in 15 mL of pyridine was added. This mixture was heated to 100 ^{0}C for 6 h., cooled to room temperature and then added to ice water, the solid separated out was filtered, air dried, and recrystallised in a suitable solvent.

General procedure for synthesis of Liquid Crystalline Polysiloxane:

Equimolar Polymethylhydrosiloxane (PMHS, Fluka-U.S.A., Mol Wt. 2262) and liquid crystalline alkene were dissolved in 20 mL toluene. This solution was bubbled by argon for 30 min. at 80 ^{0}C. The hexa-chloro platinic acid (200ug) was added and the test tube capped and heated for 20 h. at 80 C. The reaction mixture was cooled to room temperature and then added to 100 mL methanol. The solid separated was filtered and redissolved in dichloromethane and precipitated in methanol. This process was repeated for five times. The LCPS thus obtained was dried in air and then under vacuum for 12 h. Using above procedures following monomeric liquid crystals were synthesised.

1. nButenyloxy—⬡—C(=O)—O—⬡—⬡—OCH₃

2. nButenyloxy—⬡—C(=O)—O—⬡—⬡—CN

3. nButenyloxy—⬡—⬡—C(=O)—O—⬡—OCH₃

4. nButenyloxy—⬡—⬡—C(=O)—O—⬡—CN

5. nButenyloxy—⬡—⬡—COO—⬡—⬡—OCH₃

6. CH₂=CH—CH₂—O—⬡(OCH₃)—OOC—⬡—⬡—C₂H₅
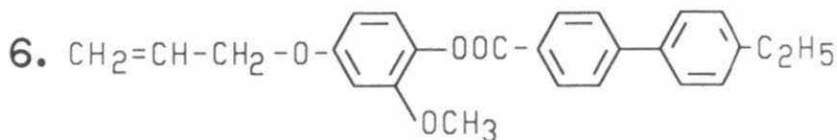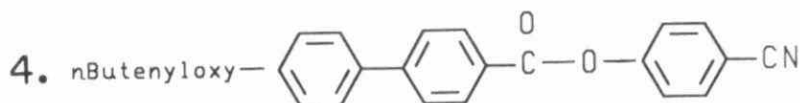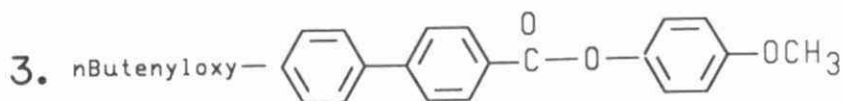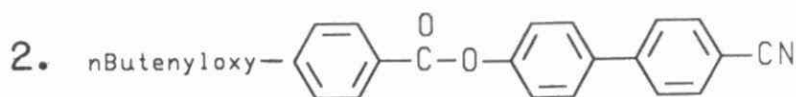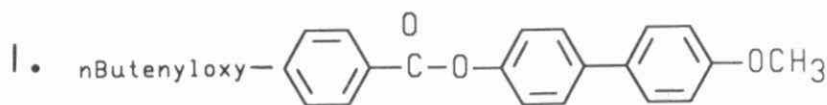
LIQUID CRYSTALLINE MONOMERS

83

Column preparation:

Fused silica capillary tubing (20 m X 0.320 mm I. D., Polymicro Technologies, Phoenix, AZ, U.S.A.) was washed by passing 10 ml methanol then purged by helium at 100 °C for 2 h. Fused silica tubing was then statically coated at room temperature using 0.3 % (w/v) stationary phase solution in methylene chloride, that was filtered through a siantered glass filter. The thickness of the film was ca. 0.2 um. The column was then conditioned by heating from 40 to 280 °C at 2 °C/min. The column was then tested for its chromatographic performance using standard mixtures and environmental samples.

Results and discussion:

The molecular weight of the liquid crystalline polysiloxane obtained from monomer (#6) was ca. 15,582. The melting point of monomer was 120°C, no transition temperature was observed using the capillary melting point method. However, LCP from this monomer melts at 80 °C and gives a clear isotropic state above 260 °C. It is always very difficult to find accurate transition temperatures but based on gas chromatographic properties of stationary phases transition temperatures can be predicted. Conversion of the monomer into poly siloxane was further confirmed by NMR spectra of both the monomer and polymer.

Chromatographic performance of the LCPS fused silica capillary column was investigated using different environmental samples and standards. GC-ECD traces of a PCDDs reference standard obtained on a LCPS column developed in this study and on a conventional DB-5 column are shown in Figure 2. The LCPS column shows better separation inspite of shorter column length. A comparable column efficiency was shown by the LCPS column to that of the DB-5 column.

We have already shown that the other type of liquid crystal column has superior selectivity for separation of 2,3,7,8-TCDD (14). The difficulty with that column was a very long retention time (ca. 100 min.) for elution of OCDD/OCDF (10). The LCPS developed in this study have comparable retention times to that of the DB-5 column in addition to better separation.

Comparison of separation of an Ontario fly ash extract on the LCPS and DB-5 column is shown in figure 3. It has been shown that a 60 m DB-5 column shows little separation of OCDD/OCDF (15). Similar separation is

84
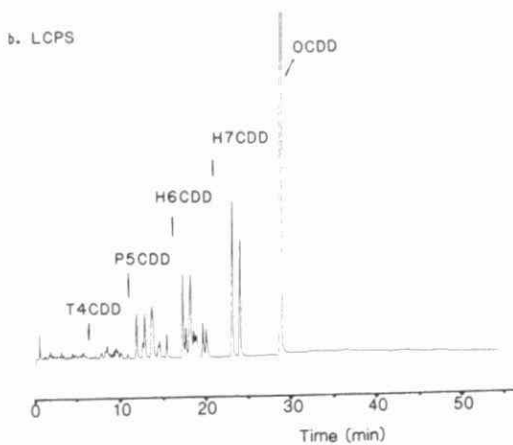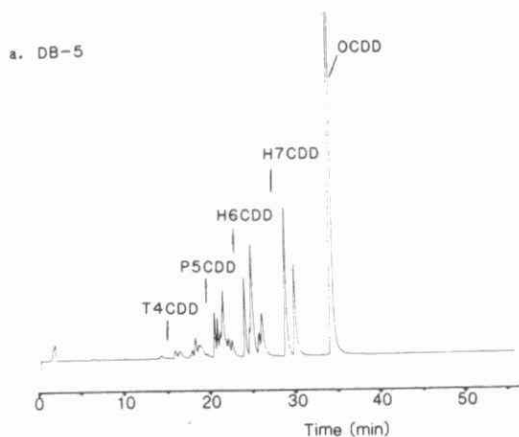
Figure 2.
GC-ECD traces of PCDD reference standard, a: column, DB-5, 30 m X 0.32 mm
I. D. fused silica capillary, temperature program 80 °C for 1 min., programmed
to 230 °C at 15 °C/min., programmed to 300 °C at 3 °C/min, b: column, Liquid
crusalline polysiloxane, 20m X 0.32 mm I. D. fused silica capillary, temperature
program 150 °C for 1 min., programmed to 250 °C at 3 °C/min.

85
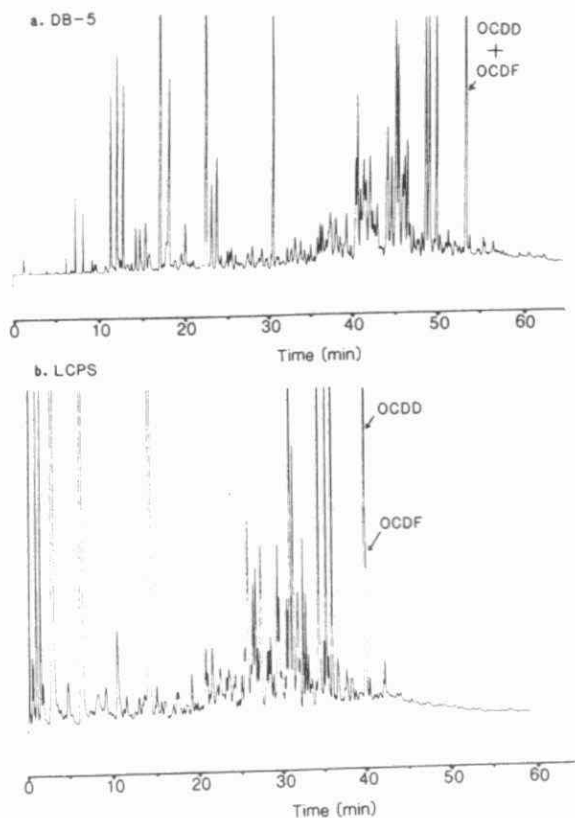
Figure 3.

GC-ECD traces of the Ontario Fly ash extrate, a: column, DB-5, 30 m X 0.32 mm I. D. fused silica capillary, temperature program 80 °C for 1 min., programmed to 230 °C at 15 °C/min., programmed to 300 °C at 3 °C/min,

b: column, Liquid crusalline polysiloxane, 20m X 0.32 mm I. D. fused silica capillary, temperature program 80 °C for 1 min., programmed to 200 °C at 5 °C/min, programmed to 250 °C at 2 °C.

achieved on the 20 m LCPS column developed in this study. It can be seen that OCDD/OCDF are not separated on a DB-5, 30 m long column.

To date, all LCPSs developed in this study have not been tested in detail as stationary phases, in particular, for isomer specific separation of 2,3,7,8-TCDD and TCDF using GC-MS/EISIM. However, those results will be included in the presentation.

References:

1.   Janini, G. M.; Muschik, G. M., Zielinski, W. L. Jr.;
      Anal. Chem. 1978, 48, 809.
2.   Zielinski, W. L. Jr., Miller, M. M., Ulma, G., Wasik, S. P.;
      Anal. Chem.   1986, 58, 2692.
3.   Naikwadi, K. P., Rokushika, S., Hatano, H., Oshima, M.;
      J. Chromatogr. 1985, 331, 69.
4.   Naikwadi, K. P., Jadhav, A. L., Rokushika, S., Hatano, H.,
      Macromol. Chem. 1986, 187, 1407.
5.   Rokushika, S., Naikwadi, K. P., Jadhav, A. L., Hatano, H.;
      HC&CC 1985, 8, 480.
6.   Rokushika, S., Naikwadi, K.P., Jadhav, A. L., Hatano, H.;
      Chromatographia 1986, 22, 209.
7.   Kuei, J. C., Tarbet, B. J., Jakson, W. P., Bradshow, J. S., Markedes, K.
      E.,   Lee, M. L.; 1985, 20, 303.
8   Apfel, M. A., Finkelmann, H., Janini, G. M, Laub, R. J., Luhmann, B. H.,
      Price, A., Roberts, W. L., Show, T. J., Smith, C. A.;
      Anal. Chem. 1985, 57, 851.
9.   Nishioka, M., Jones, B. A., Tarbet, B. J., Bradshow, J. S., Lee, M. L.;
      J. Chromatogr. 1986, 357, 79.
10.   Naikwadi K. P., McGovern, A. M., Karasek, F. W.;
      Can. J. Chem. 1987, 65, 970.
11.   Kong, R. C., Lee, M .L., Tominaga, Y., Pratap, R., Iwao, M., Castle R.
      N., Anal. Chem. 1982, 54, 1802.
12.   Markides, K. E., Nishioka, M., Tarbet, B. J., Bradshaw, J. S., Lee, M. L.;
      Anal. Chem. 1985, 57, 1296.
13.   Nishioka, M., Bradshaw, J. S., Lee, M. L., Tominaga, Y., Tedjamulia, M.,
      Castal, R. N.; Anal. Chem. 1985, 57, 309.

14.   Naikwadi, K. P., Karasek, F. W.;
      J. Chromatogr.; 1986 361, 365.
15.   Tong, H. Y., Shore, D. L., Karasek, F. W.;
      Anal. Chem.; 1984, 56, 2442.

DEVELOPMENT OF MOBILE INFRARED SPECTROSCOPY FOR
ON-SITE SPECIATION OF ORGANIC WASTES

P. Yang* and J. Osborne, Ministry of the Environment, Laboratory Services Branch, Trace Organics Section, 125 Resources Road, Rexdale, Ontario M9W 5L1, Canada

ABSTRACT

The stability and performance of three Fourier transform infrared (FT-IR) spectrometers, equipped with either conventional or corner-cube Michelson interferometer, is investigated. Evaluation of instrument stability is based on interferogram subtraction and 100% line interpretation. It is demonstrated that corner-cube retroreflectors compensate for the interferometer optical alignment and eliminate vibrational problems in mobile laboratory. In addition, FT-IR increases the field capacity and provides rapid turnaround time in the identification and classification of hazardous organic wastes.

INTRODUCTION

The mobile unit of the Ontario Ministry of the Environment is equipped with state-of-the-art analytical instrumentation to perform on-site analysis of hazardous wastes for prompt remedial action and solve environmental problems. In order to increase the field capacity in the classification and identification of hazardous organic wastes and to provide rapid turnaround time capability, the unit is investigating the suitability of a Fourier transform infrared (FT-IR) spectrometer for the mobile laboratory. In order to perform a "real world" test we have installed and tested three commercial FT-IR spectrometers in the mobile laboratory during various field investigations. The evaluation was based upon: a) "bouncing test" in which FT-IR spectrometer was shipped to the destination in the mobile laboratory and tested for the stability of the interferometer and basic optical components;

and b) the versatility of each individual data systems and
software employed to facilitate the classification and
identification of various organic wastes. It is illustrated that
infrared spectroscopy, when used concomitantly with appropriate
algorithm can fulfill the desired goals.

FT-IR SPECTROMETERS

Three FT-IR spectrometers, namely BOMEM Michelson 100,
Digilab FTS-7 and Nicolet 5DX were evaluated. The last two FT-IRs
were equipped with a conventional Michelson interferometer and
proprietary designed data system for optimized FT-IR spectral data
manipulation. The first FT-IR had a corner-cube Michelson
interferometer. An IBM PC-AT (or compatible) data station was
used to control the spectrometer and to perform the spectral data
manipulation. All three spectrometers were equipped with a 1200°K
infrared source, triglycine sulfate detector and a 16-bit
analog-to-digital converter. Software adjustable gain-ranging was
turned off in all three FT-IRs; therefore, stability evaluation
performed on these three FT-IR systems was reflecting the nature
of a corner-cube and a conventional Michelson interferometer
FT-IR.

INTERFEROGRAM SUBTRACTION

The stability and repeatability of an interferometer is best
tested using interferogram subtraction approach. It consists of
subtracting two consecutive one-scan interferograms. In theory,
difference interferogram will reveal the short term stability

problem of the interferometer, i.e., repeatability of the moving mirror drive. Therefore, a residual center burst which maintains the line shape (with either a negative going or a positive going maximum) of the original center burst and with a value <1% of the original center burst is considered to be acceptable. A residual center burst with a derivative line shape and/or a value >2% of the original center burst is an indication of a short term stability problem and should be corrected before proceeding further.

Evaluation performed on the three FT-IRs indicated no signs of such short term stability problem. In addition, values of residual interferogram obtained from the two Michelson interferometers were from 0.2 to 0.8% and was about twice as much when compared to that obtained from the corner-cube interferometer, which ranges from 0.1% to 0.35%.

HUNDRED PERCENT LINE INTERPRETATION

Hundred percent line is used to evaluate a poor mirror drive because it would produce random tilting and perturbs high frequency component more than lower frequency component; thus, result in a 100% line which deviates from the 100% transmittance. In our experiment all three FT-IRs showed no such abnormality. Furthermore, noise level obtained from a single scan, resolution 4 cm^{-1} 100% line spectrum from all three FT-IRs was about 0.18% $\pm$ 0.02% which matched well with that can be generated from a 16-bit analog-to-digital converter. It was noticed that 100% spectrum measured from the corner-cube interferometer, via ratioing single

91

beam spectra collected before and after the trip, had a deviation of about ± 20% from the 100% transmittance. We were not able to obtain a similar 100% line from the other two FT-IRs. In fact, minor optical adjustment was required to bring the two FT-IRs to the original performing standards.

SPECIATION OF ORGANIC WASTES

Hazardous waste samples normally contain one principal component along with several minor contaminants. Mandatory separation and purification is required to assure the effective use of mass spectrometry. Infrared spectrometry, on the other hand, reveals functional group information of the sample and is not subjected to the prerequisite imposed on the mass spectrometry. In addition, the availability of infrared libraries and structure prediction software make it possible for an infrared spectroscopist to classify and identify most of the incoming organic wastes. All of the above advantages make infrared spectrometry an ideal choice for on-site organic wastes speciation task and offers a fast turnaround time that cannot be matched by any other existing analytical techniques.

CONCLUSIONS

Evaluation performed in the laboratory shows that FT-IR is a valuable tool for on-site organic waste speciation. The corner-cube interferometer FT-IR offers both short term and long term stability for the speciation of organic wastes. The field capacity was increased by at least a factor of 3.

D9

MOBILE LABORATORY: DEVELOPMENTAL AND REAL WORLD APPLICATION ASPECTS

D. Toner*, B. Dalton, D. Morse, K. Hom, P. Yang and J. Osborne, Ontario Ministry of the Environment, Laboratory Services Branch, Trace Organics Section, 125 Resources Road, Rexdale, Ontario, Canada, M9W 5L1

The Trace Organics Section of Laboratory Services Branch, Ontario Ministry of the Environment has developed a mobile laboratory to provide on-site qualitative and quantitative target volatile organic compounds analysis and rapid classification and/or identification of unknown hazardous wastes. State-of-the-art instrumentation such as capillary gas chromatograph, GC/MS, and Fourier Transform Infrared Spectrophotometer have been integrated into the mobile unit over the past three years. We present in this report, established criteria for effective equipment implementation and operation of the mobile laboratory. The examples for on-site surveys illustrate the versatility of such a unit in performing real-time sample analysis which would otherwise be difficult or impossible to achieve.

A mobile laboratory is useful for on-site analysis and remedial action of environmental problems, e.g. chemical spills, screening incoming organic/inorganic wastes at licensed disposal facilities and industrial effluents. Some of the advantages of the mobile laboratory include: rapid turnaround time - the time taken for the analytical result to reach the client; minimal sample handling and storage resulting in good sample integrity; classification and identification of unknown samples and establishment of client liaison which results in a clearer definition of the problem.

The success of a mobile laboratory depends upon its design, instrumentation, well defined analytical protocols, established quality contol/quality assurance procedures and qualified staff.

DESIGN CONSIDERATIONS

The Trace Organics Section's mobile laboratory was designed to be versatile. Mandatory requirements include:

Power - The mobile laboratory is equipped with separate circuits for instrumentation and utilities. Each circuit is fed by either shoreline power or on-board generators.

Instrumentation Facilities - Shock mounted platforms are provided for instrumentation to combat vibration. Steel lined cylinder compartments house four compressed gas cylinders which terminate at four locations within the mobile laboratory, with double end shut-off quick connect fittings.

Fumehood - A 150 cfm stainless steel fumehood is located at the back of the mobile laboratory, externally exhausted.

Sample and Standard Storage - The mobile laboratory is equipped with a refridgerator with separate compartments for standards and samples. Power can be AC/DC or propane.

INSTRUMENTATION

Instrumentation depends upon the nature of the investigation, e.g. organic or inorganic analysis, expected level and range of contamination and analysis of volatile or non volatile compounds. Instrumentation must be rugged, with a degree of simplicity. Vibrations during travel remain, despite shock mounting, therefore

adequate precautions must be taken to ensure instrument performance. A
`bounce test' is usually wise before equipment is accepted for use.
Maintenance of equipment can be difficult in the field due to site
location and lack of spare parts. This type of problem can be
minimized by the instrumental design.

We have successfully operated the following instruments in the
field:

1. Automated headspace analyser, coupled to a capillary gas
   chromatograph with an effluent split to a flame ionization
   detector and electron capture detector, fully computer
   controlled.

2. Automated headspace analyser, coupled to a capillary gas
   chromatography with a flame photometric detector, fully
   computer controlled.

3. A manual purge and trap analyser, coupled to a capillary
   gas chromatograph with a mass selective detector, fully
   computer controlled.

4. A Fourier transform infrared spectrophotometer, with a
   data station capable of a compound library search.

5. Various other instruments for the determination of
   flashpoint, pH and conductivity.

ANALYTICAL PROTOCOLS

The following protocol is followed for processing environmental
samples of industrial waste, chemical spills, landfill leachate and
citizen complaints in the field.

1. **Preliminary Investigation** consisting of (a) Manifest or Waste Classification, (b) Historical Data, and (c) Odour

2. **Screening Techniques:** (a) pH - industrial waste, (b) conductivity - groundwater, (c) flashpoint - flammable liquids, (d) FTIR Screening - classification of waste, (e) mass spectral search for non targeted volatiles, (f) non aqueous headspace screening, and (g) aqueous headspace dilution

3. **Routine Analytical and Confirmational Techniques:** (a) aqueous headspace for targeted volatiles, (b) soil headspace analysis for targeted volatiles, and (c) purge and trap/GC/MSD of targeted compounds. (See Appendix 1 for targeted volatile compound list)

The Ontario Ministry of the Environment mobile laboratory is equipped with state-of-the-art equipment to perform the above analyses. Complete protocol of the procedures can be found in the Ontario Ministry of the Environment methods.

QUALITY ASSURANCE / QUALITY CONTROL (QC/QA)

Mobile staff strictly follow Ontario Ministry of the Environment guidelines and protocols for QC/QA, some of which are given below:

1. Two calibrations per set of routine headspace runs (24 samples), one at the beginning and one at the end.

2. One blank per six samples.

3. Purge and Trap/MSD confirmation of targeted compounds identified by headspace/FID/ECD.

4. Random (same instrument) duplicate analysis of calibrations and samples for within run and between run (different instrument) precision.

5. Routine check for linearity with both standards and samples (multi-level calibration)

6. Use of surrogate standard (deuterated bromobenzene) for recovery check.

7. Random external laboratory (typically MOE) confirmation of results.

8. Daily control charting of all targeted compounds.

CASE STUDIES

Two case studies are presented in order to illustrate the effectiveness of mobile monitoring. They differ widely in approach and reflect a degree of versatility.

CASE 1: SURVEY OF PRIVATE WELLS IN A SMALL COMMUNITY FOR SUSPECTED PETROLEUM HYDROCARBON CONTAMINATION.

Information regarding the type and extent of contamination was desired to determine the possible source(s) of contamination and the remedial action needed. In a meeting prior to analysis, the history of the problem, and a map outlining odour complaints was furnished.

Over sixty residential wells were tested in a two week period. Analysis revealed that of twenty-three residences with a taste and

97

odour complaint, nineteen contained evidence of gasoline contamination. The benzene concentrations ranged from 5 ppb to 365 ppb. Pump-out tests, that is pumping of wells over time with interval sampling, indicated no appreciable decrease in concentration. A second pocket of tetrachloroethylene contamination affecting four residences was also discovered in an area that was historically the site of a tannery. Several potential sources of gasoline were identified.

Prior to this survey, carbon filtration units for each affected residence was being considered for remediation of the problem. This was based on routine laboratory analysis, performed prior to the mobile survey, which showed only two affected residences for the sixty-nine sampled. Remediation was later changed to a new drilled water supply and distribution system after the mobile survey.

CASE 2: ON-SITE ANALYSIS OF LIQUID INDUSTRIAL WASTE HAULERS AT AN INTERNATIONAL BORDER.

This was a joint effort between the Investigation and Enforcement Branch of the Ontario Ministry of the Environment and the Environmental Protection Agency of the United States.

Two mobile laboratories were requested for this survey to perform the following tests:

1. pH and flashpoint were used to determine classification of acidic, basic or flammable waste - typical turnaround time of <15 minutes.

2. Heavy metal and cyanide dip tests with treated paper to screen samples for further Laboratory Services Branch analysis of these parameters - typical turnaround time <15

minutes.

3. Fourier transform infrared spectroscopic analysis - used to screen samples for principle components in accordance with the manifest reported - typical turnaround time <20 minutes.

4. Headspace/capillary gas chromatograph/FID/ECD analysis- used to screen samples for volatiles, specifically chlorinated compounds.

5. Purge and Trap/capillary gas chromatograph/mass selective detector analysis - used for aqueous samples received, as another tool in conjunction with the headspace and FTIR analysis.

Over sixty legal samples were processed in three days, with strict adherence to legal protocol and continuity. In the majority of cases, manifest screening was completed before the safety inspection of the vehicle was complete.

FUTURE CONSIDERATIONS

The increasing demand for on-site analysis required for remedial action of environmental problems has resulted in progress to expand the capabilities of the mobile laboratory and meet the demands of the Ontario public. This expansion includes:

1. Increased target compound list for mass selective detection.

2. Use of retention indicies as a more confirmational analytical protocol for headspace analysis.

99

3.   Micro-sized solid phase extractions for PCB's and PAH's in aqueous and waste media.

4.   Dynamic thermal stripping for MSD analysis for PCB's and PAH's in aqueous and solid media.

5.   Dynamic headspace for quantitative analysis of volatiles in soil.

6.   Instrumental evaluation of portable X-Ray fluorescence for identification of inorganic contamination.

APPENDIX 1

TARGETED VOLATILE COMPOUNDS

Trans-1,2-Dichloroethylene
1,1-Dichloroethane
Chloroform
1,1,1-Trichloroethane
Benzene
Carbon Tetrachloride
Bromodichloromethane
Trichloroethylene
1,2-Dichloropropane
Toluene
1,1,2-Trichloroethane
Tetrachloroethylene
Chlorodibromomethane
Chlorobenzene
Ethylbenzene
Bromoform
M/P-Xylene
O-Xylene
1,1,2,2-Tetrachloroethane
Naphthalene
1,4-Dichlorobenzene
1,3-Dichlorobenzene
1,2-Dichlorobenzene

Detection Limit is 2.0 ppb for headspace and 1.0 ppb for Purge and Trap

NON ROUTINE COMPOUNDS

Sulphur Compounds
Ketones, Acetates, Esters, Alcohols
Other Aromatics - di, tri and tetra methyl benzenes
Dowtherm - mixture of biphenyl, biphenyl oxides and methyl biphenyls

D10

Regiospecific Syntheses of All Isomeric Nitrofluorenones and Nitrofluorenes by Transition Metal Catalyzed Cross Coupling Reactions

T. Iihama,[1] J.-m. Fu, M. Bourguignon, V. Snieckus*

Guelph-Waterloo Centre for Graduate Work in Chemistry, University of Waterloo, Waterloo,

Ontario, Canada N2L 3G1

INTRODUCTION

Nitro polycyclic aromatic hydrocarbons (Nitro-PAH) are environmental pollutants which have been increasingly detected in urban ambient air particulates, diesel exhaust emissions, fly ash, photocopier fluids, and cigarette smoke.[2] The accumulating evidence of the wide environmental distribution of nitro polycyclic aromatic hydrocarbons (PAH) and the discovery, in 1980, of their potent direct acting mutagenicity has prompted increased activity in the detection, identification, and quantitation of these toxic materials.[2] Of the over 200 nitro PAH which have been recognized, the nitrofluorenes constitute one of the most potent mutagenic classes whose identification and metabolism, currently under intense study,[3] require pure analytical standards.

Available methods for the syntheses of nitrofluorenes are based on classical electrophilic nitration which provides the 2-nitro isomer in good yield but which, depending on conditions, leads to mixtures of isomeric mononitro products (including 4-nitrofluorene) requiring tedious separation and purification procedures.[2] In order to provide high purity samples obligatory for analytical and biological work, we have developed and report herein a new regiospecific and convenient route to all isomeric nitrofluorenones and nitrofluorenes which is based on the Pd(0)-catalyzed cross coupling methodology for biaryls under investigation in our laboratory.[4]

RESULTS AND DISCUSSION

Treatment of phenyl boronic acid **1a** with the bromo nitrotoluenes **2a-c** under the modified Suzuki conditions[4c] afforded the methyl nitrobiphenyls **4a-c** in high yield **(Scheme 1)**. 2-methyl-2'-nitrobiphenyl **4d** was similarly prepared by coupling the 2-methylphenylboronic acid **1b** with 1-bromo-2-nitrobenzene **3**. Reaction of **4a-d** with NBS yielded the benzyl bromides **5a-d** which, when subjected to a standard two stage oxidation procedure[5] using $AgNO_3$/KOH and $KMnO_4$, provided the carboxylic acids **7a-d**. PPA cyclization led smoothly to the nitrofluorenones **8a-d**. Et_3SiH/TFA reduction[6] was found to be unsatisfactory for all except the 4-nitrofluorenone **8d** which led to the corresponding nitrofluorene **10d** in modest (39%) yield.[7]
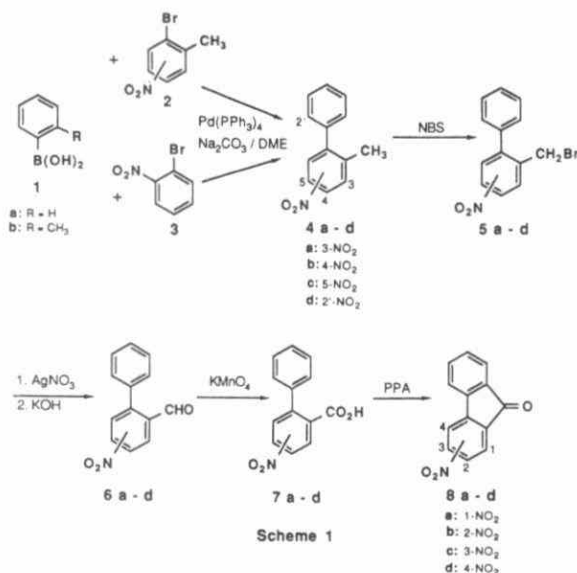
Scheme 1

To circumvent the reduction problem, the benzyl bromides **5a-d** (**Scheme 2**) were converted into the carbinols **9a-d** via the respective unisolated acetate intermediates. Direct cyclization of

104

9a-c with PPA provided the nitrofluorenes 10a-c in excellent yields while 9d led only to polymeric material. Thus although the acylium ion Friedel-Crafts cyclization of 7d occurs readily to give the fluorenone 8d, the carbenium ion derived from the corresponding carbinol 9d undoubtedly undergoes more facile intermolecular electrophilic substitution rather than intramolecular reaction into the deactivated nitrobenzene ring.

In summary, this work provides easy access to 1-, 2-, 3-, and 4-nitro-fluorenones 8a-d (31→ 46%) and -fluorenes 10a-d in (12 → 67%) overall yields. Although the route to 10d somewhat inefficient, all nitro-fluorenone and -fluorene products are obtained as single isomers whose ultra purification is not frought with the difficulties of separation of trace quantities of contaminating isomers.

5 a - d $\xrightarrow{\text{1. KOAc / HOAc}}$ 9 a - d $\xrightarrow{\text{PPA}}$ 10 a - d

1. KOAc / HOAc
2. NaOH

PPA except 9d

9 a - d
a: 3-NO$_2$
b: 4-NO$_2$
c: 5-NO$_2$
d: 2'-NO$_2$

10 a - d
a: 1-NO$_2$
b: 2-NO$_2$
c: 3-NO$_2$
d: 4-NO$_2$
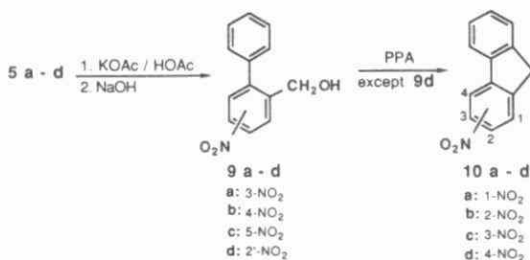
Scheme 2

MATERIALS AND METHODS

Melting points were determined on a Büchi SMP-20 melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 983 infrared spectrophotometer. ^{1}H NMR spectra were recorded on AC-200 or AM 250 spectrometers using tetramethylsilane as internal standard. Mass spectral measurements were performed by Dr. R. Smith, McMaster University, Hamilton, Ontario, Canada.

All bromonitrotoluenes and phenylboronic acid were obtained from Aldrich Chemical Co. while 1-bromo-2-nitrobenzene and 2-bromotoluene were purchased from Lancaster Synthesis Ltd.

Polyphosphoric acid (PPA) (practical grade) was purchased from Manufacturing Chemists, Inc. Dimethoxyethane (DME) was purified by distillation over CaH_2 under nitrogen. Unless otherwise indicated, standard workup is equivalent to the following operation: the reaction mixture was treated with water or saturated aq. NaCl and extracted with $CHCl_3$; the organic extract was dried with $MgSO_4$ and evaporated to dryness in vacuo.

Preparation of Methyl Nitrobiphenyls 4a-d and 6b; General Procedure:

2-Methyl-3-nitrobiphenyl (4a). To a solution of $Pd(PPh_3)_4$ (1.049 g, 0.91 mmol) in DME was added a solution of 2-bromo-6-nitrotoluene (4.054 g, 18.77 mmol) in DME (60 mL) and the mixture was stirred for 15 min under nitrogen. A solution of phenylboronic acid (3.451 g, 28.30 mmol) in EtOH (15 mL) was added, the mixture was stirred for 10 min and then treated with aqueous Na_2CO_3 (2M, 80 mL). The resulting solution was refluxed for 20 h, cooled, and the organic layer was separated. Standard workup followed by chromatography (hexane:benzene, 5:1) afforded 3.848 g (96%) of 4a, mp 72-73°C (Et$_2$O:hexane) (Lit.[8] mp 72.5-73°C); IR(CHCl$_3$) ν(max) 1528, 1353 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.35 (s, 3H, CH$_3$), 7.26-7.49 (m, 7H, ArH), 7.79 (d, J = 7.0 Hz, 1H, ArH); MS m/e (rel intensity) 213 (M, 68), 196 (100), 165 (78), 152 (43).

According to the above procedure, the following compounds were prepared:

2-Methyl-4-nitrobiphenyl (4b). 2-Bromo-5-nitrotoluene (5.016 g, 23.22 mmol), phenylboronic acid (4.232 g, 34.71 mmol), $Pd(PPh_3)_4$ (1.315 g, 1.14 mmol); yield: 93%; mp 55-56°C (Et$_2$O:hexane) (Lit.[9] mp 55-56°C); IR (Nujol) ν(max) 1515, 1344 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.36 (s, 3H, CH$_3$), 7.25-7.50 (m, 6H, ArH), 8.08 (dd, J = 8.3, 2.2 Hz, 1H, ArH), 8.14 (d, J = 2.2 Hz, 1H, ArH); MS m/e (rel intensity) 213 (M, 100), 165 (40), 152 (34).

2-Methyl-5-nitrobiphenyl (4c). 2-Bromo-4-nitrotoluene (3.956 g, 18.30 mmol), phenylboronic acid (3.360 g, 27.56 mmol), $Pd(PPh_3)_4$ (1.067 g, 0.92 mmol); yield: 99%; mp 76-77°C (Et$_2$O:hexane) (Lit.[10] mp 75.5 - 76.5°C); IR (CHCl$_3$) ν(max) 1519, 1348 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.36 (s, 3H,

CH$_3$), 7.26-7.48 (m, 6H, ArH), 8.08-8.11 (m, 2H, ArH); MS m/e (rel intensity) 213 (M, 100), 165 (59), 152 (57).

2-Methyl-2′-nitrobiphenyl (4d). 1-Bromo-2-nitrobenzene (1.010 g, 5 mmol), (2-methyl-phenyl)boronic acid[11] (0.952 g, 7 mmol) (prepared from 2-bromotoluene via metal-halogen exchange with n-butyllithium and quenched with trimethyl borate and acidic workup), Pd(PPh$_3$)$_4$ (0.289 g, 0.25 mmol); yield: 96%; mp 63.5-64.5°C (pentane) (Lit.[11] mp 63-64°C); IR (CHCl$_3$) ν(max) 1522, 1351 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.10 (s, 3H, CH$_3$), 7.08-7.35 (m, 5H, ArH), 7.47-7.67 (m, 2H, ArH), 7.96-8.01 (dd, J = 1.4, 8.0 Hz, 1H, ArH); MS m/e (rel intensity) 213 (M, 31), 196 (58), 183 (63), 165 (100), 152 (28).

2-Formyl-4-Nitrobiphenyl (6b). 2-Bromo-5-nitro-benzaldehyde (1.150 g, 5.00 mmol), phenyl-boronic acid (0.854 g, 7.00 mmol), Pd(PPh$_3$)$_4$ (0.231 g, 0.20 mmol); yield: 95%; mp 74-74.5°C (hexane); IR (CHCl$_3$) ν(max) 1691, 1525; 1347 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.40-7.69 (m, 6H, ArH), 8.45-8.50 (dd, J = 2.5, 8.4 Hz, 1H, ArH), 8.85 (d, J = 2.4 Hz, 1H, ArH), 10.00 (s, 1H, CHO); MS m/e (rel intensity) 227 (M, 85), 180 (46), 152 (100); HRMS Calcd: 227.0583. Found: 227.0579.

Preparation of Bromomethyl Nitrobiphenyls 5a-d; General Procedure:

2-Bromomethyl-3-Nitrobiphenyl (5a). A solution of 2-methyl-3-nitrobiphenyl (0.382 g, 1.79 mmol), N-bromosuccinimide (0.333 g, 1.87 mmol), and a few crystals of benzoyl peroxide in CCl$_4$ (15 mL) was refluxed (36 h), cooled, treated with benzene (50 mL), and the whole was filtered and evaporated to dryness in vacuo. The crude material was chromatographed (hexane:EtOAc, 20:1) to give 0.490 g (94%) of **5a**, mp 72.5-73°C (Et$_2$O:hexane); IR (Nujol) ν(max) 1516, 1354, 1223 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.71 (s, 2H, CH$_2$), 7.54-7.38 (m, 7H, ArH), 7.96-7.88 (m, 1H, ArH); MS m/e (rel intensity) 293 (M + 1, 2), 291 (M - 1, 2), 212 (61), 166 (63), 165 (100); HRMS Calcd: 290.9895. Found: 290.9892.

Using the above procedure, the following compounds were prepared:

2-Bromomethyl-4-Nitrobiphenyl (5b). 2-Methyl-4-nitrobiphenyl (4.313 g, 20.23 mmol), NBS (3.788 g, 21.28 mmol); yield: 73%; mp 88-88.5°C (Et$_2$O:hexane); IR (Nujol) ν(max) 1505, 1347 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.46 (s, 2H, CH$_2$), 7.40-7.55 (m, 6H, ArH), 8.18 (dd, J = 2.4, 8.5 Hz, 1H, ArH), 8.41 (d, J = 2.4 Hz, 1H, ArH); MS m/e (rel intensity) 293 (M + 1, 16), 291 (M - 1, 16), 212 (63), 166 (100), 165 (74); HRMS Calcd: 290.9895. Found: 290.9889.

2-Bromomethyl-5-Nitrobiphenyl (5c). 2-Methyl-5-nitrobiphenyl (0.416 g, 1.95 mmol), NBS (0.365 g, 2.05 mmol); yield: 76%; mp 59-59.5°C (Et$_2$O:hexane); IR (Nujol) ν(max) 1516, 1354 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.44 (s, 2H), 7.4-7.5 (m, 5H, ArH), 7.70 (d, J = 8.5 Hz, 1H, ArH), 8.14 (d, J = 2.4 Hz, 1H, ArH), 8.20 (dd, J = 2.4, 8.5 Hz, 1H, ArH); MS m/e (rel intensity) 293 (M + 1, 7), 291 (M - 1, 7), 212 (59), 166 (78), 165 (100); HRMS Calcd: 290.9895. Found: 290.9881.

2-Bromomethyl-2'-Nitrobiphenyl (5d). 2-Methyl-2'-nitrobiphenyl (0.411 g, 1.93 mmol). NBS (0.358 g, 2.01 mmol); yield: 75%; mp 83-83.5°C (Et$_2$O:hexane) (Lit.[12] mp 77-79°C); IR (Nujol) ν(max) 1517, 1354 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.17 (d, J = 10.4 Hz, 1H, CH$_2$Br), 4.41 (d, J = 10.4 Hz, 1H, CH$_2$Br), 7.10 (dd, J = 1.3, 7.5 Hz, 1H, ArH), 7.20-7.70 (m, 6H, ArH), 8.04 (dd, J = 1.2, 8.1 Hz, 1H, ArH); MS m/e (rel intensity) 293 (M + 1, 1), 291 (M - 1, 1), 212 (40), 166 (100), 165 (65).

Preparation of Formyl Nitrobiphenyls 6a-d; General Procedure:

2-Formyl-3-Nitrobiphenyl (6a). To a solution of 2-bromomethyl-3-nitrobiphenyl (5a) (1.069 g, 3.66 mmol) in dioxane (50 mL) was added a solution of silver nitrate (2.555 g, 15.04 mmol) in water (5 mL) and the whole was stirred at room temperature for 18 h. The reaction mixture was filtered, the residue was washed with EtOAc, the filtrate was treated with water, and the organic layer was separated. The aqueous layer was extracted with EtOAc (2 x 70 mL) and the combined organic layer was subjected to standard work up. Chromatography (hexane-EtOAc, 15:1 → 10:1)

afforded 0.773 g (77%) of **2-nitratomethyl-3-nitrobiphenyl** which was used without further purification. To a solution of this material (0.673 g, 2.46 mmol) in dioxane (40 mL) was added a solution of KOH (2.257 g, 40.20 mmol) in water. The mixture was stirred at room temperature for 20h, poured into water (50 mL), and the resulting solution was treated with saturated aq NaCl. The whole was extracted with CH_2Cl_2 (3 x 50 mL) and the extract was dried ($MgSO_4$) and evaporated in vacuo. Chromatography (hexane:EtOAc, 10:1) furnished 0.518 g (93%) of **6a**, mp 77.5-78°C (Et_2O:hexane); IR (Nujol) ν(max) 1705, 1528, 1345 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.29-7.35 (m, 2H, ArH), 7.43-7.50 (m, 3H, ArH), 7.65-7.74 (m, 2H, ArH), 7.88-7.95 (m, 1H, ArH), 10.20 (s, 1H, CHO); MS m/e (rel intensity) 227 (M, 1), 152 (100); HRMS Calcd: 227.0583. Found: 227.0586.

2-Formyl-4-Nitrobiphenyl (6b). 2-Bromomethyl-4-nitrobiphenyl (1.847 g, 6.32 mmol); AgNO$_3$ (4.401 g, 25.91 mmol); KOH (5.419 g, 96.59 mmol); yield: 82%. This compound was shown (mp, ^{1}H NMR) to be identical with a sample prepared by the cross coupling procedure described above.

2-Formyl-5-Nitrobiphenyl (6c). 2-Bromomethyl-5-nitrobiphenyl (0.227 g, 0.78 mmol), AgNO$_3$ (0.552 g, 3.25 mmol), KOH (0.630 g, 11.23 mmol); yield: 71%; mp 100-107°C (Et_2O:hexane); IR (Nujol) ν(max) 1686, 1520, 1343 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.35-7.56 (m, 5H, ArH), 8.17 (d, J = 8.4 Hz, 1H, ArH), 8.25-8.35 (m, 2H, ArH), 10.0 (s, 1H, CHO); MS m/e (rel intensity) 227 (M, 92), 226 (M - 1, 78), 180 (30), 152 (100); HRMS Calcd: 227.0583. Found: 227.0586.

2-Formyl-2′-Nitrobiphenyl (6d). 2-Bromomethyl-2′-nitrobiphenyl (0.372 g, 1.27 mmol), AgNO$_3$ (0.886 g, 5.21 mmol), KOH (1.128 g, 20.11 mmol); yield: 69%; mp 72-73°C (Et_2O:hexane); IR (Nujol) ν(max) 1690, 1520, 1268, 1246 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.2-7.8 (m, 6H, ArH), 7.9-8.15 (m, 2H), 9.86 (s, 1H, CHO); MS m/e (rel intensity) 227 (M, 1), 181 (100), 152 (100); MS(CI) m/e 245 (M$^+$ + NH$_4$).

Preparation of Carboxy Nitrobiphenyls 7a-d; General Procedure:

2-Carboxy-3-Nitrobiphenyl (7a). To a solution of 2-formyl-3-nitrobiphenyl (**6a**) (0.4754 g, 2.09 mmol) in acetone (20 mL) was added a solution of $KMnO_4$ (0.498 g, 3.15 mmol) in water (25 mL) and the mixture was stirred at room temperature for 4 h. It was treated with 5% aq Na_2SO_3 (50 mL), acidified (conc HCl), and the whole was extracted with $CHCl_3$ (3 x 50 mL). The organic extract was washed with water (50 mL) and extracted with 10% aq NaOH (2 x 30 mL). The alkaline extract was washed with $CHCl_3$ (30 mL), acidified (conc HCl with ice), and the resulting solution was extracted with $CHCl_3$ (3 x 30 mL). The organic layer was washed with water (50 mL), dried ($MgSO_4$), and evaporated to dryness to give 0.436 g (86%) of **7a**, mp 203-203.5°C (CH_2Cl_2:hexane) (Lit.[13] mp 200.5-201.5°C); IR (Nujol) ν(max) 1702, 1537 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3-7.7 (m, 7H, ArH), 8.12 (dd, J = 1.5, 7.9 Hz, 1H, ArH); MS m/e (rel intensity) 243 (M, 96), 226 (15), 196 (23), 195 (27), 152 (95), 115 (100).

According to the above procedure, the following compounds were prepared:

2-Carboxy-4-Nitrobiphenyl (7b). 2-Formyl-4-nitrobiphenyl (0.227 g, 1.00 mmol), $KMnO_4$ (0.237 g, 1.50 mmol); yield: 72%; mp 172.5-174°C (CH_2Cl_2:cycloxhexane) (Lit.[14] mp 173°C); IR ($CHCl_3$) ν(max) 3425 (br), 1710, 1524, 1346 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.32-7.46 (m, 5H, ArH), 7.58 (d, J = 8.5 Hz, 1H, ArH), 8.38-8.43 (dd, J = 2.4, 8.5 Hz, 1H, ArH), 8.80 (d, J = 2.4 Hz, 1H, ArH); MS m/e (rel intensity) 243 (M, 100), 226 (37), 152 (29).

2-Carboxy-5-Nitrobiphenyl (7c). 2-Formyl-5-nitrobiphenyl (0.109 g, 0.48 mmol), $KMnO_4$ (0.116 g, 0.74 mmol); yield: 98%; mp 180-181°C (CH_2Cl_2:hexane) (Lit.[15] mp 180°C); IR (Nujol) ν(max) 3200, 1688, 1521, 1308 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3-7.5 (m, 5H, ArH), 8.10-8.35 (m, 3H, ArH); MS m/e (rel intensity) 244 (M + 1, 15), 243 (M, 100), 226 (35), 152 (47).

2-Carboxy-2′-Nitrobiphenyl (7d). 2-Formyl-2′-nitrobiphenyl (0.191 g, 0.84 mmol), $KMnO_4$ (0.205 g, 1.29 mmol); yield: 90%; mp 170-170.5°C (CH_2Cl_2:hexane) (Lit.[16] mp 168°C); IR (Nujol) ν(max) 3411, 1690, 1677, 1517, 1345 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.20-7.25 (m, 2H, ArH), 7.40-7.65 (m, 4H, ArH), 8.05-8.15 (m, 2H, ArH); MS m/e (rel intensity) 243 (M, 1), 197 (100), 152 (18).

Preparation of Nitrofluorenones 8a-c; General Method:

1-Nitrofluorenone (8a). A mixture of 2-carboxy-3-nitrobiphenyl (7a) (0.383 g, 1.58 mmol) and polyphosphoric acid (6.126 g, 18.10 mmol) was heated at 160°C for 5 h, cooled to room temp, and poured into a mixture of 10% aq NaOH in ice (50 mL). Standard workup followed by chromatography (PhH) gave 0.221 g (62%) of **8a**, mp 189-189.5°C (CH_2Cl_2:hexane) (Lit.[13] mp 188.5-189.5°C); IR (Nujol) ν(max) 1716, 1532, 1360 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.39 (ddd, J = 7.3, 6.3, 2.3 Hz, 1H, ArH), 7.5-7.7 (m, 5H, ArH), 7.76 (dd, J = 7.1, 1.4 Hz, 1H, ArH).

The following compounds were prepared according to the general method given above:

2-Nitrofluorenone (8b). 2-Carboxy-4-nitrobiphenyl (0.153 g, 0.60 mmol), PPA (2.360 g, 7.00 mmol); yield: 66%; mp 222-223°C (CH_2Cl_2:hexane) (Lit.[13] mp 219-220°C); IR ($CHCl_3$) ν(max) 1724, 1528, 1340 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.41-7.63 (m, 3H, ArH), 7.77-7.81 (m, 1H, ArH), 7.93-8.05 (m, 1H, ArH).

3-Nitrofluorenone (8c). 2-Carboxy-5-nitrobiphenyl (0.100 g, 0.41 mmol), PPA (1.512 g, 4.47 mmol); yield: 88%; mp 235-235.5°C (CH_2Cl_2:hexane) (Lit.[13] mp 232°C); IR (Nujol) ν(max) 1708, 1527, 1350 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.43 (ddd, J = 7.1, 7.1, 1.7 Hz, 1H, ArH), 7.55-7.8 (m, 3H, ArH), 7.81 (d, J = 8.0 Hz, 1H, ArH), 8.20 (dd, J = 8.0, 1.9 Hz, 1H, ArH), 8.35 (d, J = 1.9 Hz, 1H, ArH).

4-Nitrofluorenone (8d). 2-Carboxy-2'-nitrobiphenyl (0.165 g, 0.68 mmol), PPA (2.477 g, 7.33 mmol); yield: 77%; mp 174-174.5°C (CH$_2$Cl$_2$:hexane) (Lit.[17] mp 172.5-173°C); IR (Nujol) ν(max) 1723, 1521, 1306 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.4-7.65 (m, 3H, ArH), 7.79 (dd, J = 7.3, 1.3 Hz, 1H, ArH), 7.95 (dd, J = 7.3, 1.1 Hz, 1H, ArH), 8.00-8.05 (m, 2H, ArH).

Preparation of Hydroxymethyl Nitrobiphenyls 9a-d; General Procedure:

2-Hydroxymethyl-5-Nitrobiphenyl (9c). A solution of 2-bromomethyl-5-nitrobiphenyl (0.747 g, 2.55 mmol and KOAc (0.512 g, 5.22 mmol) in HOAc (10 mL) was refluxed for 4 h, cooled, and treated with water (50 mL). The mixture was extracted with CHCl$_3$ (3 x 50 mL) and the organic layer was successively washed with water (50 mL) and saturated aq NaHCO$_3$ (50 mL), dried (MgSO$_4$), and evaporated to dryness in vacuo. The crude **2-acetoxymethyl-5-nitrobiphenyl** was dissolved in an EtOH (5 mL)-THF (15 mL) mixture, 1N aq NaOH (3.8 mL) was added, and the mixture was stirred at room temp. for 12 h. Standard workup and chromatography (hexane-EtOAc, 4:1 → 3:1) afforded 0.523 g (89%) of **9c**; oil; IR (neat) ν(max) 3401, 1522, 1348 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.03 (br, 1H, OH), 4.69 (s, 2H, CH$_2$), 7.25-7.50 (m, 5H, ArH), 7.79 (d, J = 8.5 Hz, 1H, ArH), 8.11 (d, J = 2.4 Hz, 1H, ArH), 8.21 (dd, J = 2.4, 8.5 Hz, 1H, ArH); MS m/e (rel intensity) 229 (M, 66), 165 (100), 152 (76).

According to the above method, the following compounds were prepared:

2-Hydroxymethyl-3-Nitrobiphenyl (9a). 2-Bromomethyl-3-nitrobiphenyl (0.803 g, 2.75 mmol), KOAc (0.542 g, 5.52 mmol), 1N NaOH (4.2 mL, 4.2 mmol); yield: 87%; mp 66-66°C (Et$_2$O:hexane); IR (Nujol) ν(max) 3572, 1527, 1360 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 3.05 (br, 1H, OH), 4.57 (s, 2H, CH$_2$), 7.42-7.54 (m, 6H, ArH), 7.63 (dd, J = 1.3, 7.7 Hz, 1H, ArH), 7.93 (dd, J = 1.3, 8.1 Hz, 1H, ArH); MS m/e (rel intensity) 229 (M, 5), 182 (41), 165 (38), 152 (100).

2-Hydroxymethyl-4-Nitrobiphenyl (9b). 2-Bromomethyl-4-nitrobiphenyl (0.611 g, 2.09 mmol), KOAc (0.415 g, 4.22 mmol), 1N NaOH (3.2 mL, 3.2 mmol); yield: 96%; oil; IR (neat) ν(max) 3395, 1516, 1344 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.05 (br, 1H, OH), 4.68 (s, 2H, CH$_2$), 7.30-7.50 (m, 6H, ArH), 8.17 (dd, J = 2.2, 8.4 Hz, 1H, ArH), 8.48 (d, J = 2.2 Hz, 1H, ArH); MS m/e (rel intensity) 229 (M, 62), 211 (31), 181 (23), 165 (100), 152 (68).

2-Hydroxymethyl-2′-Nitrobiphenyl (9d). 2-Bromomethyl-2′-nitrobiphenyl (0.265 g, 0.91 mmol), KOAc (0.180 g, 1.84 mmol), 1N NaOH (1.4 mL, 1.4 mmol); yield: 95%; mp 84-84.5°C (Et$_2$O:hexane) (Lit.[12] mp 80-82°C); IR (Nujol) ν(max) 3238, 1520, 1344 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.74 (br, 1H, OH), 4.46 (br, 2H, CH$_2$), 7.10 (dd, J = 1.0, 7.5 Hz, 1H, ArH), 7.20-7.70 (m, 6H, ArH), 7.99 (dd, J = 1.2, 8.0 Hz, 1H, ArH); MS m/e (rel intensity) 229 (M, 10), 198 (25), 182 (76), 152 (82), 166 (100).

Preparation of Nitrofluorenes 10a-c; General Procedure:

3-Nitrofluorene (10c). A mixture of 5-nitro-2-hydroxymethylbiphenyl (9c) (0.088 g, 0.383 mmol) and PPA (1.764 g, 5.2 mmol) in CHCl$_3$ (5 mL) was refluxed for 26 h, cooled, and treated with a slurry of 10% aq NaOH (30 mL) and ice. The whole was extracted with CHCl$_3$ (3 x 40 mL) and subjected to standard workup to give, after chromatography (hexane-EtOAc, 20:1 → 10:1), 0.068 g (84%) of 10c, mp 105-106°C (CH$_2$Cl$_2$:hexane) (Lit.[18] mp 103-106°C). If CHCl$_3$ was omitted, the yield of 10c was lower (35%); IR (neat) ν(max) 1524, 1338 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 3.99 (s, 2H, CH$_2$), 7.35-7.5 (m, 2H, ArH), 7.55-7.6 (m, 1H, ArH), 7.65 (d, J = 8.3 Hz, 1H, ArH), 7.85-7.9 (m, 1H, ArH), 8.18 (dd, J = 8.3, 2.2 Hz, 1H, ArH), 8.58 (d, J = 2.2 Hz, 1H, ArH).

1-Nitrofluorene (10a). 3-Nitro-2-hydroxymethylbiphenyl (0.108 g, 0.47 mmol), PPA (1.444 g, 4.5 mmol); yield: 86% without CHCl$_3$ as solvent; mp 106-106.5°C (CH$_2$Cl$_2$:hexane) (Lit.[18] mp 104-106°C); IR (Nujol) ν(max) 1518, 1338 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.39 (s, 2H, CH$_2$), 7.3-7.65 (m, 4H, ArH), 7.8-7.85 (m, 1H, ArH), 8.06 (d, J = 7.5 Hz, 1H, ArH), 8.16 (d, J = 8.2 Hz, 1H, ArH).

2-Nitrofluorene (10b). 4-Nitro-2-hydroxymethylbiphenyl (0.095 g, 0.41 mmol); PPA (1.394 g, 4.13 mmol); $CHCl_3$ as solvent; yield: 79% (without $CHCl_3$, yield: 55%); mp 154-154.5°C (CH_2Cl_2:hexane) (Lit.[19] mp 156°C); IR (Nujol) ν(max) 1515, 1334 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.01 (s, 2H, CH$_2$), 7.4-7.45 (m, 2H, ArH), 7.6-7.65 (m, 1H, ArH), 7.8-7.9 (m, 2H, ArH), 8.30 (dd, J = 8.4, 2.0 Hz, 1H, ArH), 8.39 (d, J = 2.0 Hz, 1H, ArH).

4-Nitrofluorene (10d). A solution of **8d** (0.093 g, 0.41 mmol) and triethylsilane (0.142 g, 1.22 mmol) in CF$_3$COOH (4 mL) was stirred at room temp for 2 d. Standard workup and chromatography (hexane:PhH, 3:1 → 2:1) furnished 0.034 g (39%) of **10d**, mp 76-76.5°C (CH$_2$Cl$_2$:hexane) (Lit.[17] mp 75-76°C); IR (Nujol) ν(max) 1521, 1347 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 3.98 (s, 2H, CH$_2$), 7.35-7.45 (m, 3H, ArH), 7.55-7.6 (m, 1H, ArH), 7.75 (dd, J = 7.5, 0.9 Hz, 1H, ArH), 7.86 (d, J = 8.0 Hz, 1H, ArH), 8.0-8.1 (m, 1H, ArH).

References

1. Visiting Scientist, Nippon Soda Co. Ltd., Kanagawa, Japan.

2. For an excellent perspective of the analytical, chemical, synthetic, and biological aspects of nitro-PAH, see White, C.A. ed., **Chromatographic Methods: Nitrated Polycyclic Aromatic Hydrocarbons**, Huthig Verlag, Heidelberg, **1985**.

3. Rickert, D.E. ed., **Toxicity of Nitroaromatic Compounds**, Hemisphere Publishing Co., Washington, **1985**; Ball, L.M.; Kohan, M.J.; Williams, K.; Nishioka, M.G.; Gold, A.; Lewtas, J. In **Polynuclear Aromatic Hydrocarbons**; Cooke, M.; Dennis, A.J. Eds. Batelle, Press, Columbus, OH, **1988**, 59.

4. a) Snieckus, V.; Alves, T. Tetrahedron Lett., **1988**, 29, 2135; b) Sharp, M.J.; Cheng, W.; Snieckus, V. Tetrahedron Lett., **1987**, 28, 5093; c) Cheng, W.; Snieckus, V. Tetrahedron Lett., **1987**, 28, 5097; d) Sharp, M.J.; Snieckus, V. Tetrahedron Lett., **1985**, 26, 5997.

5. Letsinger, R.; Jamison, J. J. Am. Chem. Soc., **1961**, 83, 193.

6. West, C., Donnelly, S., Kooistra, D., Doyle, M. J. Org. Chem. **1973**, 38, 2675.

7. Of the other isomers, **8a** yielded a mixture, **8b** gave starting material (32%) and carbinol **i** (64.5%), and **8c** afforded nitrofluorene **10c** (14%) and carbinol **i** (73%). Further reduction of the 1- and 3-nitro carbinols **i** under the Et_3SiH or $NaBH_4$/THF (Gribble, G., Kelly, W., Emery, S. Synthesis, **1978**, 763) conditions gave starting material and dimer **ii**. Reduction of 1-nitroflurenone (($Et_3SiH/TiCl_4$) gave carbinol **i** and the dimer **iii**.

i   ii   iii
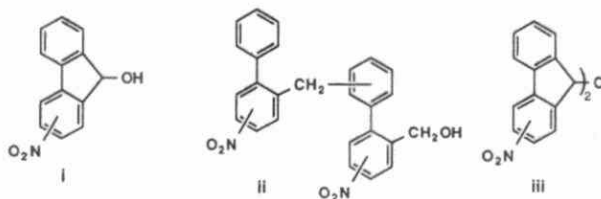
8.   Hoegerle, K.; L'Écuyer, P. Can. J. Chem. 1959, 37, 2068.

9.   Melby, R.; Brown, R.K.; Sandin, R.B. J. Am. Chem. Soc. 1950, 72, 1032.

10.   Henry, D.W. J. Heterocyclic Chem. 1966, 3, 503.

11.   Miller, R.B.; Dugar, S. Organometallics, 1984, 3, 1261.

12.   Muth, C.W.; Ellers, J.C.; Folmer, O.F. J. Am. Chem. Soc. 1957, 79, 6500.

13.   Chase, B.H.; Hey, D.H. J. Chem. Soc. 1952, 553.

14.   Heilbron, I.M.; Hey, D.H.; Wilkinson, R. J. Chem. Soc. 1938, 113.

15.   Earl, R.F.; Barrick, J.G. J. Am. Chem. Soc. 1948, 70, 1492.

16.   Bell, F. J. Chem. Soc. 1934, 835.

17.   Weissburger, J.H.; Weissburger, E.K.; Morris, H.P. J. Am. Chem. Soc. 1952, 74, 4540.

18.   Yost, Y.; Gutmann, H.R. J. Chem. Soc. Sect. C. Org. Chem. 1969, 345.

19.   Mittheilung, I. Chem. Ber., 1901, 34, 1758.

D11

PREPARATION OF HETEROCYCLIC POLYNUCLEAR AROMATIC

COMPOUNDS AS ANALYTICAL STANDARDS

E. Lee-Ruff* and B.E. George

Department of Chemistry,
York University,
Toronto, Ontario M3J 1P3

The widespread occurrence and toxicological properties of polynuclear aromatic hydrocarbons (PAH) has promoted extensive studies of this class of compounds. Recently interest has focussed on PAH compounds incorporating one or more cyclopentene rings[1-3] after the observation that cyclopenta-[c,d] pyrene (CPP) is a common environmental contaminant and exhibits a high level of mutagenic and carcinogenic activity[4]. The more simple analogues of CPP aceanthrylene (1) and acephenanthrylene (2) were also recently synthesized [2,5-9] and found to exhibit mutagenic activity[5,10]. Aceanthrylene (1) is found to be more potent than 2 and this behaviour has been correlated with the stabilization energies associated with the suspected carbocation intermediates produced from the epoxide metabolites[10]. Sulfur containing polynuclear aromatic compounds (PAC's) have been also detected in environmental samples derived from organic combustion sources.[11] Most of these sulfur PAC's are in the form of PAH's with one or more of the benzene rings replaced by thiophenes.

| 1 | 2 | 3 | 4 | 5 |
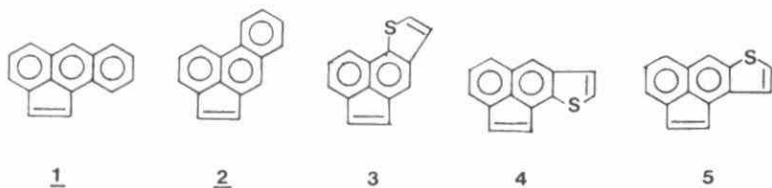
In contrast to the carbocyclic systems relatively little is known about the biological properties of sulfur heterocyclic analogues of mutagenic and carcinogenic PAH's because of the lack of standards, although certain sulfur analogues of carcinogenic PAH's have been shown to be even more potent than the parent PAH.[12]

We have developed a program towards the synthesis of heterocyclic analogues of caracinogenic PAH's and have recently reported the preparation of acenaphtho[5,4-b]thiophene (3)[13] an analogue of acephenanthrylene (2). Two thiophene isomers of aceanthrylene are possible, Acenaptho[3,4-b]thiophene (4) and acenaphtho[4,3-b]thiophene 5. Various perhydro derivatives of 4 and 5 have been reported in certain crude oil distillates[14,15] but to our knowledge the parent heterocycles are unknown. In this note we would like to describe the synthesis of 4.

119

Results and Discussion

Attempts at preparing **4** and **5** using a two carbon substituted precursor derived from a napthothiophene failed to undergo ring formation of the ace-bridge. The method that was adopted was along similar lines to Plummer's synthesis of aceanthrylene[9]. Reaction of naphtho[2,3-b]thiophene (**6**) readily prepared by our reported route involving a cyclobutane rearrangement[16], was treated with oxalyl chloride and anhydrous aluminum chloride to give the diketone **7** in 52% yield (Scheme 1) along

Scheme 1
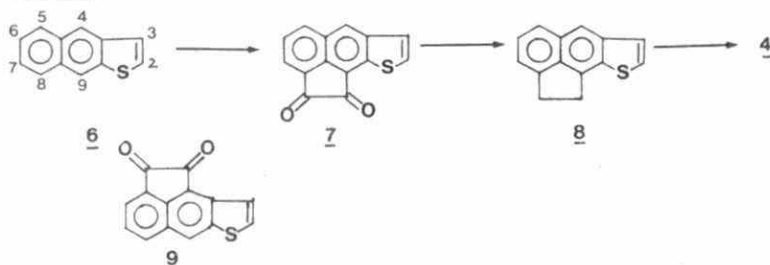
with polar products which were not identified. The acequinone bridge was assigned the 8-9 bonding rather than 5-4 (**9**) on the basis of NMR experiments which indicated long range coupling between proton 3 (AB pattern) and 4 broad singlet) as well as small (2%) NOE enhancements observed in selective irradiations of these nuclei. This assignment was further substantiated by similar NMR data obtained for **4**. Attempts to assign the regiochemistry of the quinone bridge in **7** using selectively monodeuterated 4-d **6** and 9-d **6** obtained in an earlier study[16] and reacting these with oxalyl chloride under the same conditions gave the diketone **7** with scrambling of the deuterium in both cases. The regiochemistry of acylation is somewhat unusual in that acylations of naphtho and benzothiophenes normally occur at C-2 of the thiophene ring. For instance Castle and Lee have reported that naphtho[2,3-b]thiophene (**6**) undergoes acylation with phthalic anhydride to give the C-2 acylated product.[17] The unidentified polar products associated with the formation of **7** in the oxalyl chloride reaction of **6** may be associated with mono-acylations at C-2 and C-3 as well as other positions in the ring. The

absence of 5-4 bridged quinones cannot be readily explained. A proximal sulfur atom may be responsible in directing bis acylations to the 8-9 positions of 6.

Reaction of diketone 7 with hydriodic acid resulted in deoxygenation to acenaphthene[3,4-b]thiophene (8) in 76% yield. This unusual reaction is related to Harvey's observations of polycyclic aromatic quinones being readily reduced with HI to the fully aromatic systems.[18] The dehydrogenation of 8 to the aromatic thiophene was readily accomplished with 2,3-dichloro-5,6-dicyano-benzoquinone in 70% yield. The yellow crystalline product exhibited a proton nmr spectrum consistent with the substitution pattern (see Figure 1). Two sets of AB signals are discernable and centered at 7.7 and 7.25 ppm. The AB signal centered at 7.25 ppm is assigned to the ace-bridge protons and those at 7.7 ppm to the thiphene ring protons on the basis of comparison with those reported for aceanthrylene[6] and naphtho[2,3-b]thiophene.[16] Further support for the structure assignment of 4 was obtained from the 2-D homonuclear shift correlated spectrum and selective homonuclear NOE difference experiments. Irradiation of the singlet peak at 8.4 ppm (H-4) led to signal enhancements of the doublet peak at 7.94 ppm (H-5) and 7.64 ppm (H-3, one-half of AB centered at 7.7 ppm). Irradiation of the doublet signal at 7.4 ppm (H-8, one-half of AB centered at 7.25 ppm) led to enhancements of the doublet signal at 7.81 ppm (H-7) and 7.1 ppm (H-9, one-half of AB centered at 7.1 ppm). The combined COSY and NOE difference spectra permit the assignment of all of the proton signals for 4 which are detailed in the experimental section. The uv/visible spectra of 4 resembles very closely that for aceanthrylene 1 (see Figure 2) indicating the similarity of the π-electronic framework.

Work is in progress to determine the mutagenic activities of thiophenes 3 and 4.

Experimental Section

Melting points (mp) were determined on a Reichert melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded on a Unicam SP-1000 instrument as thin films or KBr pellets. Ultraviolet (UV) spectra were measured on a Unicam SP800-A spectrometer and Hewlett Packard 8451A diode array spectrophotometer. Proton NMR spectra were recorded on a

Bruker AM-300 (300 MHz) and AM-500 spectrometers using samples dissolved in $CDCl_3$ containing 1% Me_4Si as internal standard. All NMR values are reported as chemical shift δ in ppm downfield from Me_4Si. Mass spectra were recorded on a V.G. Micromass 16F spectrometer. High resolution mass spectrometry was performed at the McMaster Regional Centre for Mass Spectrometry using a VG ZAB-E instrument in the El mode at 70eV. Elemental analyses were performed by Guelph Chemical Laboratories Limited. Naphtho[2,3-b]thiophene was prepared by a four step route described by us.[16]

8,9-Acenaphtho[2,3-b]thiophenequinone(7).

A solution of naphtho[2,3-b]thiophene (6)[16] (0.178 g, 1 mmole) in dichloromethane (10 ml) was added slowly to a stirred mixture of oxalyl chloride (0.35 ml, 4 mmoles) and anhydrous aluminum chloride (0.23 g, 1.7 mmoles) in dry dichloromethane (20 ml) at 0-5 °C. Stirring was continued at 0 °C for 6 h. The black solution was poured onto ice/water and filtered. The orange solution was washed with saturated Na_2CO_3 (15 ml), water (2 x 15 ml), dried over anhydrous $MgSO_4$ and filtered. The filtrate was evaporated to give a residue which was chromatographed on a column (flash grade silica gel) using 1:1 petr. ether: dichloromethene as the eluting solvent. This gave a red crystalline material (0.120 g, 51.7%) 7; mp 282-283 °C; ^{1}H NMR $(CDCl_3)$: δ 8.8 (S, 1H, H-4), 8.28 (d, J=8.5 Hz, 1H, H-5), 8.25 (dxd, J=5.5, 0.7 Hz, 1H, H-3), 8.07 (d, J=6.8 Hz, 1H, H-7), 8.0 (d, J=5.5 Hz, 1H, H-2), 7.78 (dxd, J=8.5, 6.8 Hz, 1H, H-6); IR (KBr) 1680 cm^{-1} (C=O); uv (CH_2Cl_2) λmax 266 nm (log ϵ = 4.36), 366 nm (log ϵ = 3.52), 386 nm (log ϵ = 3.51); mass spectrum, m/e (relative intensity) 238 (58, M$^+$), 210 (100, M-CO), 182 (78, M - 2(CO)); high resolution MS. calc. for $C_{14}H_6O_2S$ m/e 238.0099; Found: 238.0094; Anal. Calcd for $C_{14}H_6O_2S$: C, 70.65; H, 2.54; S, 13.47; Found: C, 70.83; H, 2.47; S, 13.19;

8,9-Acenaphthene[2,3-b]thiophene 8.

A mixture of quinone 7 (120 mg, 0.5 mmole) and 47% hydriodic acid (20 mmole) in acetic acid (5 ml) was heated to reflux for 20 h. The hot solution is then poured into a 1% aqueous sodium bisulfite solution (50 ml). The solution was extracted with dichloromethane (2 x 20 ml) and the combined organic extracts were washed with water (20 ml), saturated bicarbonate solution (2 x 20 ml) and dried over anhydrous $MgSO_4$, filtered and evaporated to give an oil which was applied to a column of flash grade silica gel. A

1:1 dichloromethene: pet. ether solution eluted a yellow oil **8** (80 mg,
76%); ^{1}H NMR (CD_2Cl_2): δ 8.18 (S, 1H, H-4), 7.67 (d, J=8.3 Hz, 1H, H-5),
7.51 (d, J=5.7 Hz, 1H, H-3 or H-2), 7.45 (dxd, J=6.9, 8.3 Hz, 1H, H-6), 7.39
(d, J=5.7 HJz, 1H, H-2 or H-3), 7.25 (d, J=6.9 Hz, 1H, H-7), 3.65
(t, AA'BB', 5=7.2 Hz), 2H, methylene H's), 3.55 (t, AA'BB', J = 7.2 H_2, 2H,
methylene H's). This product undergoes slow oxidation in air to give **7** as
one of the products so that no satisfactory elemental analysis could be
obtained.

Acenaphtho[3,4-b]thiophene 4.

A solution of **8** (80 mg, 0.38 mmole) and DDQ (216 mg, 0.95 mmole) in 30
ml of toluene was heated to reflux for 30 minutes. The slurry was cooled,
concentrated by rotovap and the residue was applied to a column containing
basic alumina and eluted with toluene. An orange crystalline material **4** was
obtained (0.057 g, 70%); mp 74-75 ^{o}C; ^{1}H NMR ($CDCl_3$): δ 8.35 (s, 1H, H-4),
7.9 (d, J=8.2 Hz, 1H, H-5), 7.76 (d, J = 6.7 Hz, 1H, H-7), 7.7 (d, J = 5.6
Hz, 1H, H-2), 7.62 (dxd, J = 5.6, 0.6 H_2, 1H, H-3), 7.57 (dxd, J = 8.2, 6.7
Hz, 1H, H-7), 7.37 (d, AB, J = 5.2 Hz, 1H, H-9), 7.13 (d, AB, J = 5.2 Hz,
1H, H-8); mass spectrum, m/e (relative intensity) 208 (100, M$^+$), 160 (18),
128 (21); hgh resoluton MS, calc. for $C_{14}H_8S$ m/e 208.0367, found 208.0357.
Anal. Calcd for $C_{14}H_8S$: C, 80.77; H, 3.85; S, 15.38; Found: C, 81.05; H,
3.97; S, 15.26; uv (EtOH) λmax 258 nm (log ε = 4.23), 350 nm (log ε = 3.87).

References and Footnotes

1.   Sangaiah, R.; Gold, A.; Toney, G.E.;Toney, S.H.; Claxton, L..; Easterl-
     ing, R.; Nesnow, S. Mutat. Res. (1983), **119**, 259.
2.   Chung, Y.S.; Kruk, H.; Barizo, O.M.; Katz, M.; Lee-Ruff, E. J. Org.
     Chem. (1987), **52**, 1284.
3.   Cho, E.P.; Harvey, R.G. Tet. Letters (1987), **28**, 861.
4.   Eisenstadt, E.; Gold, A. Proc. Natl. Acad. Sci. U.S.A. (1978), **75**,
     1667.

123

5. Amin, S.; Balanikas, G.; Huie, K.; Hussain, N.; Geddie, J.E.; Hecht, S.S. J. Org. Chem. (1985), **50**, 4642.

6. Becker, H.D.; Hansen, L.; Andersson, K. J. Org. Chem. (1985), **50**, 277.

7. Scott, L.T.; Reinhardt, G.; Roelofs, N.H. J. Org. Chem. (1985) **50**, 5886.

8. Sangaiah, R.; Gold, A. Org. Prep. Proced. Int. (1985), **17**, 53.

9. Plummer, B.F.; Al-Saigh, Z.Y.; Arfan, M.J. J. Org. Chem. (1984), **49**, 2069.

10. Kohan, M.J.; Sangaiah, R.; Ball, L.M.; Gold, A. Mutat. Res. (1985), **155**, 95.

11. Grimer, G.; Jacob, J.; Naujack, K.W.; Dettbarn, G. Anal. Chem. (1983), **55**, 892.

12. Croisy, A.; Mispelter, J.; Lhoste, J.M.; Zajdela, F.; Jacquignon, P. J. Heterocyclic Hem. (1984), **21**, 353.

13. Lee-Ruff, E.; Chung, Y.S. J. Heterocyclic Chem. (1986) **23**, 1551.

14. Lyapina, N.K.; Perfenova, M.A.; Nikitina, T.S.; Brodski, E.S.; Ulendeeva, A.V. Neftekhimya (1980), **20**, 747; C.A. **94** 105951e.

15a. Karmanova, L.P.; Lyapina, N.K.; Frolova, K.L.; Shmakov, V.S.; Poberii, A.Y. Neftekhimya (1985), **25**, 115; C.A. **102**, 169283e.

15b. Lumpkin, H.E. Anal. Chem. (1964), **36**, 2399.

16. Lee-Ruff, E.; Ablenas, F.J. Can. J. Chem. (1987), **65**, 1663.

17. Tedjamulia, M.L.; Tominaga, Y.; Castle, R.N.; Lee, M.L. J. Heterocyclic Chem. (1983), **20**, 1143.

18a. Konieczny, M.; Harvey, R.G. J. Org. Chem. (1979), **44**, 4813.

18b. Konieczny, M.; Harvey, R.G. Org. Snyth. (1984), **62**, 165.

Figure 1

¹H NMR Spectrum (300 MHz) of Acenaphtho[3,4-b]thiophene **4**

125
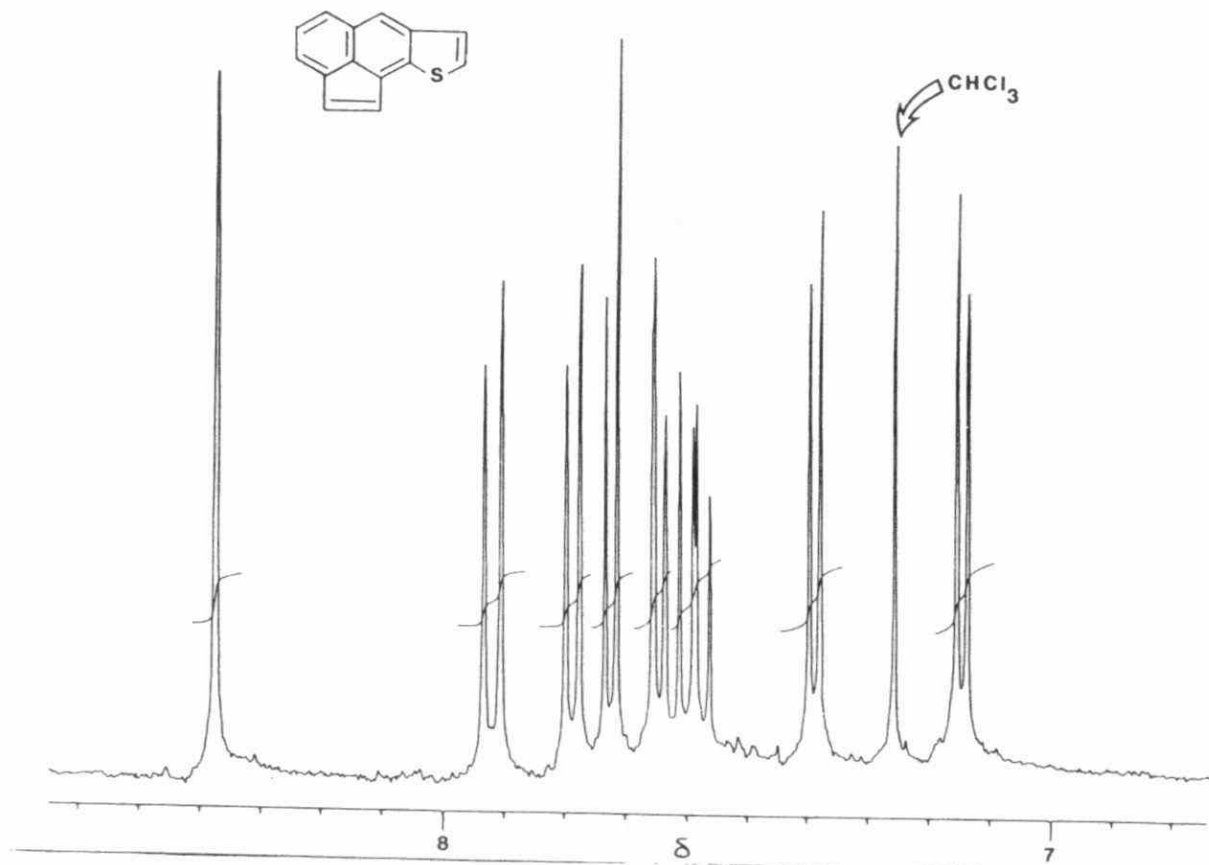
Figure 2

UV Spectra of Acenaphtho[3,4-b]thiophene (**4**) and Aceanthrylene in CH_2Cl_2

D12
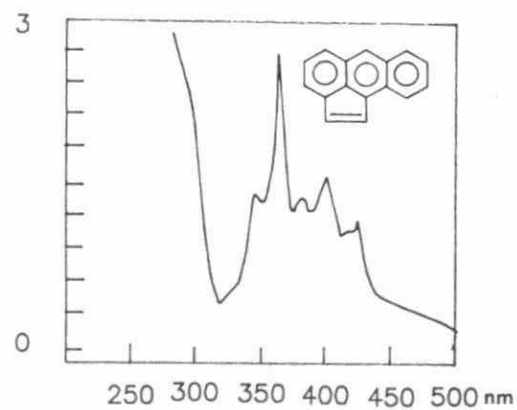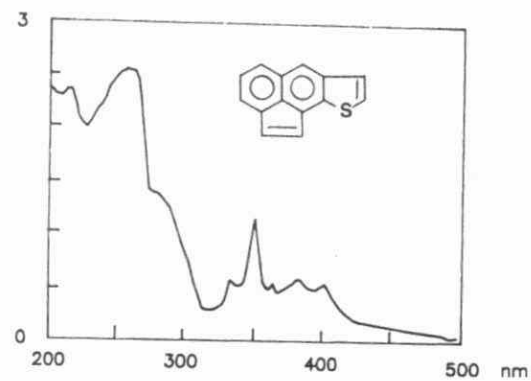
APPLICATION OF ICP SPECTROMETRY IN HEALTH AND ENVIRONMENT: A CASE STUDY OF SOIL
INGESTED BY CHILDREN.

Xioaru Wang, Alexandra Lasztity, Miklos Viczian, Yescheskel Israel, and Ramon M.
Barnes

University of Massachusetts, Department of Chemistry, GRC Towers, Amherst, MA
01003-0035

Knowing the amount of non-food materials like dust, paint or soil that a
child ingests incidentally or purposely can aid in identification of sources of
environmental poisons and often is needed in making environmental risk assessment
evaluations. Establishing whether or not a child ingests non-food materials is one
requirement, identifying which of a number of non-food materials contributed to a
child's illness is another, and demonstrating how much non-food is ingested is yet
another. Either alone or in combination inductively coupled plasma atomic emission
(ICP-AES) and mass spectrometry (ICP-MS) have been evaluated and applied in ad-
dressing these requirements. Two specific situations have been considered: (i)
identifying environmental sources resulting in lead poisoning, and (ii) quantify-
ing daily average soil ingestion by toddlers during normal play.

The identification of environmental sources of lead poisoning is based upon
the determination of the isotopic composition of stable lead in a child's blood
and in environmental samples of dust, paint, soil, or water. The statistical co-
incidence of lead isotope ratios in blood and the suspect environmental sample
provides a means for sorting and identifying sources. Additionally lead concentra-
tions in these samples can be determined concurrently by on-line isotope dilution.
ICP-MS provides a rapid, routine measurement for both lead isotope ratio and lead
concentrations in these samples. Examples of this application will be presented.

Among the approaches possible in developing a measurement strategy to quan-
tify a child's daily soil ingestion is the determination of innocuous metals pre-
sent both in soils and in the child's excreta but not in food. Selection of suit-
able elements is based upon their concentration in soil and their detectability in
food, feces and urine. In a recent investigation involving 64 children eight ele-
ments were determined for 68 dust, 2600 fecal, 1800 food, and 180 soil samples.
Major elements like Si were determined by ICP-AES, while trace elements like Zr
must be measured by ICP-MS. Sample treatment and preparation to avoid contamina-
tion and losses becomes the primary concern, once measurement accuracy and preci-
sion are confirmed. Novel sampling problems involved with commercial disposable
diapers compounded fecal analysis, and the contribution of trace metals in urine
to diaper samples was documented. Interpretation of data is complicated by the
bioavailability of some test elements, and recovery of well-characterized soil fed
to adult volunteers was measured. The individual contributions of dust and soil to
the total intake can be distinguished in some situations. Finally, a preliminary
study to apply lead isotope ratios as a tracer for soil ingestion has been un-
dertaken.

D13

A NOVEL PROBE DESIGN FOR THE DIRECT INSERTION OF SOLID SAMPLES IN THE
INDUCTIVELY COUPLED PLASMA FOR ANALYSIS BY ATOMIC EMISSION AND MASS
SPECTROMETRY.

L. Blain and E.D. Salin# , Department of Chemistry, McGill University,
801 Sherbrooke St. W., Montreal, Quebec, Canada H3A 2K6.

INTRODUCTION

Solid samples still represent a tough challenge for the analyst's skills

and resources, particularly in fields of application such as metallurgy,

geology and environmental monitoring. The initial interest in direct

solid analyses grew out of a dissatisfaction with the lenghty sample

preparation procedures of conventional methods. Although the rate

limiting step will probably remain the sample preparation step, there is

a clear need for a more rapid alternative to these dissolution and

preconcentration procedures. Not only are these techniques time

consuming, but there is also an acute risk of contamination by

impurities in the reagents used, and in an age of parts per billion

(ppb) and sub-ppb detection limits, even the purest of reagents can

become a significant source of contamination.

The feasibility of the direct insertion of powdered solid samples

in the inductively coupled plasma (ICP) for their analysis by atomic

emission spectrometry (AES) has been demonstrated (1,2,3). The principal

advantages of this technique are the simplicity of the equipment needed

(4), the very limited sample preparation that it requires (1) and its

excellent detection limits (2). Yet there is still a need for lower

detection limits, as the typical concentration of many important

elements in solids of environmental and geological interest is often

very close to some of the detection limits that have been reported.

One sure way to improve detection limits is to increase the instantaneous concentration of analyte in the source per unit time. With the DSID this means either increasing the mass of sample in the probe or facilitating the transfer of heat from the plasma to the sample in the probe. We felt that we could achieve both goals by pressing the sample directly into a pellet, rather than packing it inside the graphite or metal cups that are conventionally used (1,2,3,8). So far, we have successfully pelletized g-standards with graphite in ratios of 1:10 to 1:2 and they did not disintegrate upon their insertion in the plasma. We have established some preliminary detection limits for eight elements by atomic emission spectrometry (AES) and found that the technique has to be further optimized before yielding all of its potential advantages. We have also established a detection limit for lead by mass spectrometry (MS) with this technique, corresponding to 0.16ppb.

EXPERIMENTAL

ICP-AES DSID

The difficulties involved with recording transient signals have been highlighted in the literature (4,5,6). We used a THERMO JARRELL-ASH ICAP 61 direct reading spectrometer equipped with a fast spectrum shifter, to preserve the full multi-element capacity of ICP and to allow the measurement of the background around the lines of interest. Apart from its obvious advantages, the multi-element feature of ICP-AES allows the implementation of internal standards which are often necessary to keep the precision of geological analyses within reasonable bounds . The very rapid spectrum shifter of the THERMO JARRELL-ASH direct reader is

necessary in cases where the emission spectrum of a sample is complex
and requires extensive spectral corrections (6).

Early in our investigations we found that the instrument's
software did not accommodate transient signals very well and we chose to
write our own operating software, and we have already described a
prototype version of that program elsewhere (7). The latest program
stores the data bytes returned by the spectrometer's controller in a
binary record, rather than a text file. The data can then be processed
first by a program that calculates the net intensities from the data
bytes, then finds the best background correction data and fits a cubic
spline through it to interpolate the background at the on-line positions
which is then directly subtracted from the measurements at these
positions. Another program is then used for graphics display and a semi-
automatic peak finding procedure is used to calculate the five peak
parameters :

    - begining time of the peak
    - time at which the peak maximum occurs
    - ending time of the peak
    - maximum intensity (peak height)
    - integrated intensity (peak area)

which are then stored in a text file for later use.

The conditions under which the ICP was run are listed in Table II.
These conditions were used because they have been reported by various
workers as optimal (1,2), but some recent experiments have confirmed our
expectation that two sets of parameters will need to be optimized
carefully, namely the plasma's applied power and the combination of
insertion and viewing heights.

Initially we used graphite electrodes such as those of arc AES as
supports for the pellets but we quickly found then impractical (7). Our

experience with the wire loop DSID (4,5) immediately suggested an
alternate support (Figure 1) for the pellet. This support is lighter
than the previous design, so that the pellet can be inserted deeper in
the discharge without extinguishing it, and it is less bulky. The latter
results in less heat dissipation down the support and less chance of
pushing the plasma onto the sides of the torch. The stepper motor driven
shaft on which this DSID probe assembly rests is described in detail
elsewhere (4).

The preparation of the pellets is straightforward, a suitable
weight of SPEX g-standards is ground in proportions varying from 1:5 to
1:15 with spectroscopic grade graphite powder, for about 20 minutes in a
mixer/mill. This mixture is then weighed out into 5 aliquots of about
260 mg each. These are pelletized with a manual press of the kind used
in bomb calorimetry. After a few tries one quickly develops a feel for
the pressure needed, and it is then possible to press uniform and firm
pellets that do not break down when they are inserted into the plasma.
Further details on the reagents, the mixer/mill and the press can be
found in Table I and in ref. 7.

ICP-MS DSID

The instrument used for the DSID-ICP-MS experiments was a Perkin
Elmer SCIEX ELAN 250, its operating conditions are listed in Table III.
The insertion depth and the sampling position were roughly optimized at
the start of the experiments using the maximization of raw ion counts as
optimization criterion. Although we had a good idea of what to expect
for the time profile of the signals from our AES work, we preferred to

restrict ourselves to a single mass channel to avoid missing any important temporal features, especially at the lower concentrations. We chose to look at lead because it is well behaved temporally, and it is a priority pollutant that still had an unsatisfactory detection limit by DSID-ICP-AES. We felt that it would best highlight the greater sensitivity of ICP-MS (3).

Our access to the ICP-MS was restricted, so we proceeded with the acquisition of preliminary calibration data without thoroughly optimizing the system. We chose compromise conditions, suitable for the determination of heavy elements, based on our previous experience on this instrument with the wire loop DSID (4).

0.5 mm
Tungsten wire

Figure 1.

RESULTS AND DISCUSSION

ICP-AES DSID

The signals observed for the insertion of pellets are transient
peaks very much like those of the conventional DSID. For the same
concentration of analyte in the sample, the pellets' signal is more
intense and lasts much longer. Figure 2 shows the net signal we observed
for the insertion of 30mg of a 5% copper in graphite mixture with a
conventional DSID probe, and by contrast, Figure 3 is the net background
corrected signal recorded for the insertion of a 250mg pellet of the
same mixture. This increase of the signal cannot be explained in terms
of the greater sample mass alone. Hence, the greater surface area of
sample directly exposed to the source must also contribute to improving
the transport efficiency of the analyte into the atomization zone of the
plasma.

The same type of volatility categories that have already been
reported (1,2) were observed, where the volatile elements such as
mercury and lead have peak-shaped signals of relatively short duration
that appear early after the insertion, while the more refractory
elements result in broader humps that rise quickly and decay rather
slowly. It was also found that the signal of the very refractory
elements, such as titanium, and those that form refractory carbides,
such as molybdenum, tungsten and vanadium, is often not significantly
different from the surrounding background.

A set of compromise conditions that would allow the estimation of the standard figures of merit of this technique was used. A set of sixteen elements was picked to cover the range of possible signals :

  1)- the very short peaks   : Hg, Pb, Se & Sn
  2)- those of intermediate duration : As, Cu, Mg & Zn
  3)- those of long duration   : Fe & Ni
  4)- the weak signals   : Co, Cr, Mn, Mo, V & W

With an integration time of 5 seconds the signal of all sixteen elements could be recorded adequately at concentrations of 1 ppm, 3.3 ppm and 10 ppm. Furthermore, we could also record the signal of about half the elements at a concentration of 100 ppm.

The calibration curves for As, Cu, Hg, Mg, Pb, Se and Sn were linear up to 100 ppm. The signals of the other elements lasted longer than the observation period at that concentration. This sets a practical limit on the dynamic range of the technique for a one-shot multi element analysis, which can be circumvented by replicate determinations with different dilution factors of the sample in graphite or different integration times. To keep things simple for these preliminary investigations, the maximum concentration was limited to 10 ppm. At this stage, the primary concern is improving the detection limits of the technique, so that our interest lies mainly with the weak emitters. Certain weak emitters such as Cd, Hg and Pb, are often found in ultra-trace levels and are also of great concern to many users. Twelve elements of the set have linear calibration curves with slopes significantly different from zero, and they are listed in Table IV along with their detection limits.

These results are for an unoptimized system and should be improved by a more intelligent choice of plasma forward power, insertion depth, probe geometry and viewing parameters. Even as they are, these detection limits compare well with those already reported in the literature (1&2).

The signals for Fe, Mo, V and W were all very weak, and even at 100 ppm they were not significantly different from the baseline, the detection limits for these elements are thus meaningless and do not appear in Table IV. This has already been observed (1,2) and has been explained by the refractory character of the compounds these elements are either present as (1) or of the compounds they form with the surrounding graphite diluent (2). Apparently, this kind of matrix effect has already been successfully minimized by a proper choice of matrix modifier or volatilization enhancer (2,8).

ICP-MS DSID

We have successfully interfaced our pellet DSID with the ELAN ICP-MS and the results are impressive. The absolute detection limit for lead as its oxide in the solid was 2 pg in a 250 mg pellet of 3/16" diameter. The slope of the calibration curve was obtained with standards that were made up by diluting a 1 ppm g-standard 1:20 in graphite, thus the detection limit in concentration units for lead in the g-standards was 0.16 ppb. Initially the conventional position of the sampling cone inside the plasma was used, and the ion counts that were measured were very low, even at concentrations as high as 100 ppb lead in graphite. A slight axial offset of the sampler from the center of the plasma

138

resulted in increases of the ion counts by four to six orders of magnitude.

The greater sensitivity of ICP-MS puts very stringent requirements on the purity of the graphite diluent used in the preparation of the pellet. It also imposes more strict sample preparation protocols to minimize the possibility of contamination by the grinder's mixing chamber and the press's punch and die set. About half of the graphite blanks were severely contaminated at the detection levels of ICP-MS when the mixer/mill's mixing chamber was simply cleaned with a brush and soapy water, followed by an ethanol rinse and a purge with compressed air. On the other hand, when a new mixing chamber was used to prepare a series of graphite blanks, the baseline was featureless for all five replicates.

The baseline was systematically offset from zero by a few hundred counts, so the total observation period was set to last approximately twice the duration of the peak. Furthermore, the data acquisition was synchronized with the insertion of the probe in the plasma to ensure a reproducible appearance time for the peak, early on in the observation period. The average number of counts in the latter half of the signal, after the peak had returned to baseline, was then used as an estimate of the baseline under the peak. The ion counts were numerically integrated over the first half of the measurement period to determine the total analytical signal. Figure 4a) shows the average signal for 100 ppb lead in graphite, 50 ppb and clean graphite blanks. Figure 4b) is a close up of the average blank signal. The slight dip in the early part of the blank signal is due to an overcompensation by our baseline correction procedure.

CONCLUSION

Even in its initial stage of development this technique is proving itself to be very sensitive. The simplicity of preparing the probes makes it well suited for routine implementation. We are currently experimenting with various combinations of integration time, off-line positions and integration bounds to maximize the resolution of the short signals from the surrounding background noise and thus maximize the sensitivity of the measurement, while attempting to minimize the loss of information for the longer lasting signals.

The detection limits we obtained in these preliminary investigations are very encouraging, yet there are some features of the probe design that need to be improved before all its advantages can be realized. In order of importance these are : the diameter of the probe, the insertion depth and the viewing height.   A larger probe means that more sample is introduced in the plasma at once, but it probably disturbs the plasma more and may result in higher background noise. The insertion depth should also have this kind of competition between the effect on the stability of the plasma and the amount of sample introduced in the plasma. The correlation between the optimal insertion depth and the optimal viewing zone has already been reported (2), so that these two parameters must be simultaneously optimized by a multivariate technique. We will report on a comprehensive optimization of these factors in the future.

140

DSID signal with a conventional probe
30 mg of 0.5% Cu in graphite

Figure 2.

DSID signal with a pellet probe
250 mg of 0.5% Cu in graphite

Figure 3.

141

Pellet DSID signal for Pb by ICP–MS
graphite blank, 56 ppb and 116 ppb

Figure 4 a.

Blank signal for Pb by ICP–MS
250 mg pellet of pure graphite
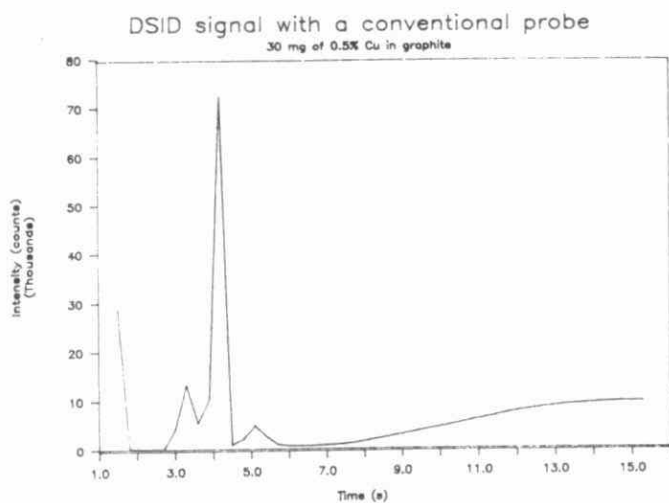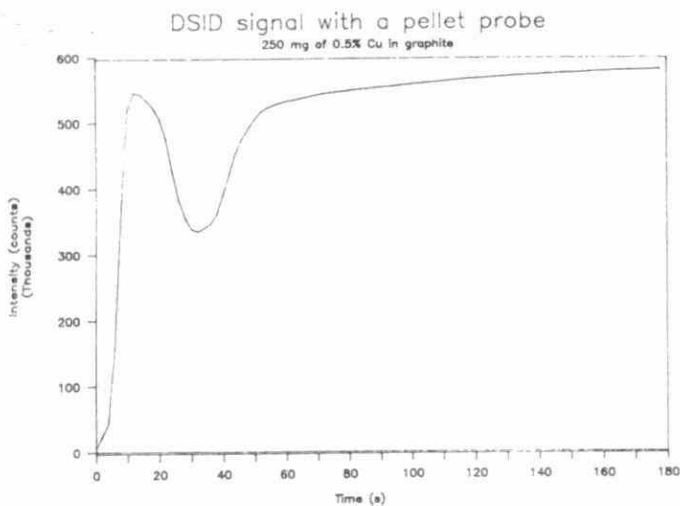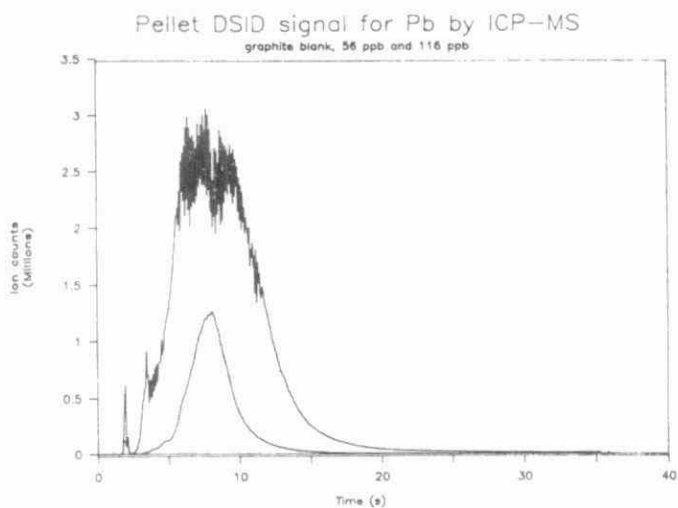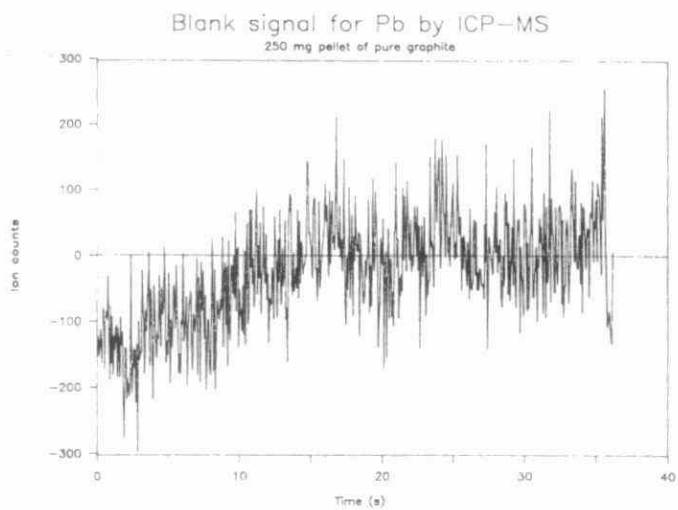
Figure 4 b.

<div align="center">

TABLE I

EQUIPMENT, REAGENTS AND SOFTWARE

</div>

ICP-AES
    Thermo Jarrell Ash ICAP 61
    from : Thermo Jarrell Ash Corporation
           Waltham, Massachusetts, U.S.A.
ICP-MS
    Perkin Elmer SCIEX ELAN 250
Mixer/mill
    5100 Spex Mixer/Mill
    Methacrylate cylinders
    5/16" Tungsten carbide balls
    Tungsten carbide lined nylon caps
    from : SPEX INDUSTRIES, Inc.
           Metuchen, New Jersey, U.S.A.
Press
    2811 Pellet press with 1/2" punch and die
    from : PARR INSTRUMENT COMPANY
           Moline, Illinois, U.S.A.
    1/4", 3/16" and 1/8" punch and die sets
    from : McGill University machine shop
Host computer
    AST 286
    from : AST RESEARCH, INC.
           Irvine, California, U.S.A.
Reagents :
    G-standards   SPEX INDUSTRIES, Inc.
                       Metuchen, New Jersey, U.S.A.
    Pelletizing graphite, "spectroscopic electrodes"
                       BAY CARBON, Inc.
                       Bay City, Michigan, U.S.A.
Software
    Turbo Pascal
    from : BORLAND INTERNATIONAL Inc.
           Scotts Valley, California, U.S.A.

TABLE I
EQUIPMENT, REAGENTS AND SOFTWARE

ICP-AES
      Thermo Jarrell Ash ICAP 61
      from : Thermo Jarrell Ash Corporation
            Waltham, Massachusetts, U.S.A.
ICP-MS
      Perkin Elmer SCIEX ELAN 250
Mixer/mill
      5100 Spex Mixer/Mill
      Methacrylate cylinders
      5/16" Tungsten carbide balls
      Tungsten carbide lined nylon caps
      from : SPEX INDUSTRIES, Inc.
            Metuchen, New Jersey, U.S.A.
Press
      2811 Pellet press with 1/2" punch and die
      from : PARR INSTRUMENT COMPANY
            Moline, Illinois, U.S.A.
      1/4", 3/16" and 1/8" punch and die sets
      from : McGill University machine shop
Host computer
      AST 286
      from : AST RESEARCH, INC.
            Irvine, California, U.S.A.
Reagents :
      G-standards   SPEX INDUSTRIES, Inc.
                             Metuchen, New Jersey, U.S.A.
      Pelletizing graphite, "spectroscopic electrodes"
                           BAY CARBON, Inc.
                           Bay City, Michigan, U.S.A.
Software
      Turbo Pascal
      from : BORLAND INTERNATIONAL Inc.
            Scotts Valley, California, U.S.A.

<div align="center">

TABLE II
ICP-AES PARAMETERS

</div>

Plasma parameters :
    Applied power   (kW) : 1.00
    Plasma gas flow   (LPM) : 15
    Auxilliary gas flow (LPM) : 2.0
Spectrometer parameters :
    Viewing height   (mm) : 15
    Integration time   (s) : 5
    Observation period (s) : 320
    2 off-line spectrum shifter positions
    average wavelength offset : 0.15nm
DSID parameters :
    Probe diameter : 3/16"
    Probe support : graphite platform
    Pre-heat step : -20 mm[1] for 10 s
    Insertion step : -2 mm[1] for 360 s
    Cooling step   : -110 mm[1] for 60 s
    (1) distance from top of load coil

<div align="center">

TABLE III
ICP-MS PARAMETERS

</div>

Plasma parameters :
    Applied power   (kW) : 0.75
    Plasma gas flow   (LPM) : 12
    Auxilliary gas flow (LPM) : 1.5
DSID parameters :
    Probe diameter : 3/16"
    Probe support : tungsten wire loop
    Pre-heat step : -20 mm[1] for 10 s
    Insertion step : -4 mm[1] for 40 s
    Cooling step   : -120 mm[1] for 40 s
    (1) distance from top of load coil
MS parameters :
    Mass channel (a.m.u.) : 208
    B ion lens setting   : 50
    P ion lens setting   : 50
    Dwell time   (ms) : 20
    Points per scan   : 3
    Scans per run   : 605

TABLE IV
DETECTION LIMITS IN PELLETS BY AES

Element	Pellet[1] (ppb)	Reference[2] (ppb)	Element	Pellet[1] (ppb)	Reference[2] (ppb)
As	47	44	Mn	19	
Co	12		Ni	139	
Cr	147		Pb	59	33
Cu	3		Se	18	
Hg	12		Sn	149	60
Mg	20		Zn	294	90

(1) 250 mg pellet
(2) 10 mg sample, reference (2)

REFERENCES

1   Brenner, I.B.; Lorber, A.; Goldbart Z. *Spectrochim.
     Acta* **87**, *428*, 219.
2   Shao, Y.; Horlick, G. *Applied Spectrosc.* **86**, *40*, 386.
3   Montaser, A.; Golightly, D.W., Eds.; *Inductively Coupled
     Plasmas in Atomic Spectrometry*; VCH Publishers Inc:
     New York, 1987.
4   Boomer, D.W.; Powell, M.J.; Sing, R.L.A.; Salin, E.D.
     Anal Chem. **86**, *58*. 975.
5   Sing, R.L.A.; Salin, E.D. paper #32, 1986 Winter
     Conference on Plasma Spectrochemistry, Kailua-Kona,
     Hawaii, Jan. 2-8, 1986
6   Taylor, P.; Schutyser, P. *Spectrochim. Acta* **86**, *418*, 81.
7   Blain, L.; Salin, E.D.; Boomer, D.W.; Powell, M.; *A
     Novel Probe Design for the Direct Insertion of Solid
     Samples in the Inductively Coupled Plasma for Analysis
     by Atomic Emission and Mass Spectrometry.* **submitted for
     publication**.
8   Nickel H.; Reisch M.; Mazurkiewicz M. paper F5.1, XXV
     Colloquium Spectroscopicum Internationale, Toronto,
     Canada, June 21-26, 1987

Analysis of Germanium and Tin by Hydride Generation D.C. Plasma Atomic Emission Spectrometry[*]

Ian D. Brindle and Xiao-chun Le

Chemistry Department, Brock University, St Catharines,

Ontario L2S 3A1, Canada

Introduction

The measurement of hydride-forming elements at low concentrations is of interest to atmospheric chemists concerned with the elemental composition of airborne particulates. Germanium is an element that is widely distributed in nature but occurs rarely in concentrated form. The association of germanium with coal dust and ash has made these materials sources of germanium. This association with coal burning may also be a benefit for in the study of airborne particulates. Thus the collection and analysis of airborne particulates on filters may show the relationship of germanium concentrations to industrial activities associated with coal-burning. Tin can appear in particulates derived particularly from scrap metal smelting.

With the development of hydride generation as an efficient sample introduction method, detection limits of 2 to 3 orders of magnitude better than conventional nebulisation methods have been achieved using these spectrometric methods coupled with hydride generation techniques.[1-4] Germanium was first determined by Pollock and West.[5] This has subsequently been improved by a number of people, most recently by Andreae[6] for sea water and by Brindle and Ceccarelli-Ponzoni.[7]

Interference has been a recurrent problem with the determination of hydride forming elements and transition elements have generally been regarded as the major source of the problem. Several workers have offered reagents to overcome some of these problems. These reagents are often specific to the particular element. Thus interferences in the determination of tin have been reduced by sodium oxalate,[8] tartaric acid,[9,10] iodide,[9] iron(III),[9] and thiourea,[11] while ortho-phosphoric acid, malic acid, and EDTA have been used in the reduction of interferences in the determination of germanium.[12]

Our previous work[13] showed that L-cystine was superior to other agents used to reduce interference from transition metal ions in the determination of arsenic. Thus L-cystine has the advantages of high efficiency, low arsenic blank, low toxicity and ready availability. With a 3% L-cystine solution in 5 M HCl, interference from 10,000⁻ g ml^{-1} of Co(II), Cu(II), Fe(III & II) and Ni(II), and 1000 µg ml^{-1} of Ag(I), Hg(II) and Pt(IV) in the determination of arsenic by hydride generation were eliminated. The use of L-cystine in the elimination of interferences suggested that, with appropriate modifications, this same system might be used in the reduction of interferences in the determination of other hydride forming elements such as germanium and tin.

Experimental Section

Apparatus

The equipment used included a Beckman Spectraspan V d.c. plasma atomic emission spectrometer with a modified sample tube, a Dataspan data storage system, a Sargent-Welch XKR chart recorder, and a Beckman hydride generator modified as described previously.[14] A further modification for this application was the incorporation of a calcium chloride drying tube instead of the Porapak Q tube. In these determinations, the presence of Porapak Q did not improve the signals. The expense of the Porapak Q and the need to replace it frequently because of wetting made it expedient to eliminate this tube, thus, two drying columns in series packed with calcium sulfate and calcium chloride respectively were used for the determination of tin and a single calcium sulfate column was used for the determination of germanium.

The emission lines at the 284.00 nm and 303.906 nm were used for tin and germanium respectively, with an entrance slit of 50 μm (horizontal), 300 μm (vertical) and with an exit slit of 100 μm (horizontal), 300 μm (vertical). Photomultiplier voltage and amplifier gain settings were used appropriately to provide convenient signals and to minimise noise. A Brinckman variable volume Macro-Transferpettor was used for all analyte injections with the volume fixed at 5.0 ml. A disposable syringe was used for the injection of 1.0 ml of 4% sodium tetrahydroborate(III) solution.

Reagents

Tin stock solution (1000 μg ml^{-1}) was prepared by dissolving 1 g tin shot weighed accurately ("Baker Analysed" Reagent, 100.0%) in concentrated hydrochloric acid (100 ml) and was diluted with deionised water. Germanium standards were prepared from ammonium germanium(IV) oxalate hydrate (99.998 % Gold Label, Aldrich Chemical Company), with a certified germanium concentration of 14.5 %. Standard solutions were made by serial dilution from these stock solutions with 0.05 M HNO$_3$ unless otherwise stated. Low concentration standards were made up immediately before use to obviate any problems with losses due to adsorption or other causes.

Sodium tetrahydroborate(III) from Anachemia was used as the reducing agent since it had a very low tin blank. Later lots were found to have higher concentrations of tin. Thus there is an obvious need to check on each batch of reagent before it is used. Relatively higher tin blanks were found in 98% NaBH$_4$ from Aesar and Aldrich corresponding to 0.1 μg g^{-1}. In order further to decrease the tin blank in the NaBH$_4$ reagent, solution were treated by sparging. An appropriate amount of NaBH$_4$ was dissolved in deionised water. This solution was sparged with argon for

149

30 minutes. The tin blank was decreased significantly by this procedure due probably to the release of the tin hydride from the $NaBH_4$ solution. An alternative method for the reduction of the tin concentration has been described by Hodge *et al.* where electrolysis was used to reduce the tin in a heavily contaminated sodium tetrahydroborate(III) reducing agent.[15] To minimise decomposition, sufficient solid sodium hydroxide was added to the solution to make it 0.1 M with respect to NaOH. This solution was filtered prior to use. Solutions of $NaBH_4$ were prepared fresh every three days and kept in polyethylene bottles prior to use.

BDH AnalaR nitric acid was found to have a high tin blank, and so E. Merck Suprapur nitric acid was used, which was found to have a low tin and germanium blank. L-cystine was obtained from Sigma (Sigma grade) and was also found to have a low tin and germanium blank. All other chemicals were analytical reagent grade or better. Tin blanks in the reagents were subtracted from tin signals, germanium blanks were found to be negligible.

Sample Dissolution
Determinations in the presence of L-cystine

NBS Standard Reference Materials, Open-Hearth Iron 55E, Low Alloy Steel 363, and Copper "Benchmark" II and III were selected for analysis as representing a range of concentrations. Approximately 0.1 to 2 g of each sample was weighed accurately. The sample was dissolved in 10 ml of 1:1 nitric acid water solution with gentle heating and then transferred to a 500 ml volumetric flask. The solution was diluted to the mark with deionised water. A 10 ml aliquot of the sample solution was transferred to a 100 ml volumetric flask and diluted to the mark with 0.04 M nitric acid solution. The final concentration of nitric acid in the analyte solution was approximately 0.05 M.

Sample preparation with L-cysteine

Two samples were prepared for determination, the National Bureau of Standards Standard Reference Materials (SRM) "Benchmark" Copper I, and Open Hearth Iron 55E.

Copper I: A sample of the SRM (1.0-1.5 g) was weighed accurately and transferred to a beaker. A solution of 1:1 V/V nitric acid (20 ml) was added and the solution was allowed to stand until all of the sample had dissolved. The solution was transferred to a 100 ml volumetric flask and made up to the mark with deionised water. A 2.0 ml aliquot was taken and diluted to approximately 85 ml with a solution of 0.4 % m/V L-cysteine. The pH was adjusted to 2.3-2.5 with a 1:1 V/V solution of ammonia before the solution was transferred to a 100 ml volumetric flask and made up to the mark with 0.4% m/V L-cysteine.

Open Hearth Iron 55E: A sample of the SRM (1-1.5 g) was weighed accurately and transferred to a beaker. A solution of 1:1 aqua regia V/V (20 ml) was added and the mixture was allowed to stand, with gentle warming, for one hour. The solution was then transferred to a 500 ml volumetric flask. After the solution was made up to the mark with deionised water, a 5.0 ml aliquot was taken and diluted to 100 ml with a 0.4 % m/V solution of L-cysteine. Adjustment of the pH to 2.3-2.5 was made with 0.5 M nitric acid prior to the solution being made up to the mark in the volumetric flask.

Duplicate samples of the two SRMs were spiked with germanium to determine recoveries. Thus, 0.5 µg germanium was added to one gram of Copper I and 20 µg germanium was added to one gram of the Open Hearth Iron 55E.

Results and Discussion

As indicated in our previous work,[16] the relative position of the plasma and the slit is an extremely important factor for the response of the instrument. This is supported by the work of Ebdon et al., [17] who, after performing a simplex optimisation procedure on the d.c. plama atomic emission spectrometer in the normal aspiration mode, observed that the "vertical viewing points" (i.e. the observation height) is critical and depends on the element being determined. A simplex optimisation was performed on the system using the hydride generation process in order to find the best conditions for the determination. Since sleeve gas pressure and horizontal position of the plasma were considered by Ebdon[17] to be least significant, only two factors, the carrier gas flow rate and the background signal (a non- linear function of observation height), were used for the simplex. Signal to background ratio was taken as the response. Using set conditions for the generation of germane (outlined below), the optimum conditions for the determination were found to have an argon flow rate of 730 ml min^{-1} and a background signal of 6800 (with no gas flowing through the hydride generator). After the simplex was performed, the two factors were varied one at a time to find the range of the optimum signal. It was found that virtually identical signals could be obtained over a range of flows of argon from 600 to 760 ml min^{-1} and a background reading of 6000 to 9000. Thus the argon flow rate of 730 ml min^{-1} and the background signal of 6800 were used for the entire study. It should be noted that the background fell to between 400 and 500 during the determination as the argon flow changed the shape of the plasma. The background signal, without gas flowing through the central tube, proved to be the best and most reproducible indicator of the observation height during the initial set up of the system.

The Hydride Generation Process

The rapidity of evolution of stannane from solution during reduction by tetrahydroborate(III)[16] suggested that similar conditions may also obtain in the generation of germane. Preliminary experiments showed that this was indeed the case and so all further determinations were performed with argon flowing continuously through the apparatus. Addition

of solid L-cystine to the reaction vessel before reduction increased the signal from tin and so addition of solid L-cystine was also considered to be important for the determination of germanium. In addition, it was felt that the soluble L-cysteine might be more easily handled and thus provide convenient interference reduction similar to the rather insoluble L-cystine which created a foaming problem.

Sodium tetrahydroborate(III) solutions with concentrations from 1 to 10% m/V were used to identify the best concentration. Within the range of 4 to 10%, results were virtually identical. Below these values, the signals were reduced. A solution of 6% sodium tetrahydroborate(III) stabilised with sodium hydroxide (0.1M) was chosen for all determinations.

Nitric acid and hydrochloric acid were investigated as media for tin and germanium solutions. The effect of their concentrations was found to be rather similar on the signals from tin and germanium in preliminary experiments and so nitric acid was chosen as the medium of choice since most samples of metals etc. are dissolved in nitric acid prior to analysis and since germanium can easily be lost from hydrochloric acid via its volatile tetrachloride. Signals from tin and germanium are considerably enhanced by the presence both of L-cysteine and of L-cystine. In addition, the best signals from these elements were achieved in the presence of 0.4% L-cysteine and at a lower acid concentration (0.01-0.02M HNO_3). Compared with the signal in the presence of only nitric acid, in the presence of 0.4 % L-cysteine, the signals increased up to approximately 100%. It is interesting to note that the pH of the solution, after the reaction is complete, is still slightly basic (pH 8.4-9.0) with L-cystine, L-cysteine, and without any interference reducing agent.

L-Cystine created a foam during the determination which limited the volume of solution that could be used for the analysis to approximately 5.0 ml. This foaming was reduced considerably when the solution was made up to 0.4% m/V with L-cysteine instead and allowed a larger volume of the analyte solution to be used. The best volume for this system was found to be 9.0 ml. The time for a complete determination typically was less than one minute.

Interference

Our previous work [13] showed that a solution of 3% L-cystine in 5 M HCl played an efficient role in reducing interferences from transition metal ions in the determination of arsenic. L-cystine dissolves in 5 M hydrochloric acid but is poorly soluble in distilled water. Clearly a strongly acidic solution would interfere with the production of both SnH_4 and GeH_4. To solve this problem initially, solid L-cystine was directly added to the reaction vessel for each determination. It rapidly became clear that L-cystine was efficient for reducing interferences from transition elements.

Preliminary studies were carried out with tin to choose the appropriate amount of L-cystine to be added to the reaction vessel. The tin signal increases with the increase of L-cystine added until 0.07 g had been added. At this point, the tin signal is approximately 10% greater than the signal in the absence of L-cystine and remains at this level as increasing amounts of L-cystine are added. In the presence of interfering ions, a greater amount of L-cystine is required. Thus in the presence of 10 μg ml^{-1} or 100 μg ml^{-1} Ni(II), the tin response increases until it reaches a maximum when 0.3 g of L-cystine is added. Although satisfactory recovery of 10 μg ml^{-1} Ni(II) is achieved at this level, in the presence of 100 μg ml^{-1} nickel, the recovery is only 60% and stays at this level over the addition of 0.3 g to 0.9 g L-cystine. Therefore, 0.4 g of solid L-cystine was the amount chosen to be added to the reaction vessel for each determination.

In the absence of L-cystine, 0.10 μg ml^{-1} Ni(II), 0.50 μg ml^{-1} Co(II), 5.0 μg ml^{-1} Cu(II) or 5.0 μg ml^{-1} Fe(II) reduce the tin signal severely. These results are in agreement with reports in the literature.[11, 11,18] However, with the addition of solid L-cystine, solutions of 10 μg ml^{-1} Ni(II), 100 μg ml^{-1} Co(II), 1000 μg ml^{-1} Cu(II) or 10000 μg ml^{-1} Fe(II) do not interfere significantly with the tin signal. Thus, with L-cystine, the high concentrations of transition metal ions can be tolerated without interference in the determination of tin by stannane generation. Compared with other interference reducing agents, such as thiourea, sodium oxalate, and iodide, L-cystine is the most efficient in reducing tin interferences from transition metal ions. Table 1 summarises the recoveries of 0.40 ng ml^{-1} tin in the presence of other interfering ions with and

without the addition of L-cystine.

Table 1
Recovery of 0.40 ng ml^{-1} from Interfering Ions

Interferant	Form of Interferant	Interferant Concentration (mg ml^{-1})	Recoveries % without L-Cystine	with L-Cystine
Ag(I)	AgNO$_3$	10	96	98
Au(III)	AuCl$_3$	1	100	100
		10	87	77
		100	17	46
Cd(II)	Cd(NO$_3$)$_2$	100	71	100
		1000	83	74
Cr(VI)	K$_2$Cr$_2$O$_7$	100	102	100
		1000	76	82
Hg(II)	HgCl$_2$	10	98	100
		100	91	102
		1000	80	96
Mn(II)	MnSO$_4$	1000	97	100
		10000	100	100
Mo(VI)	(NH$_4$)$_6$Mo$_7$O$_{24}$	100	81	98
		1000	42	67
		10000	22	36
Pb(II)	Pb(NO$_3$)	100	100	-
		1000	103	105
Pd(II)	PdCl$_2$	1	65	58
		10	48	21
		100	6	11
Pt(IV)	H$_2$PtCl$_6$	1	89	63
		10	46	37
		100	7	26
V(V)	NH$_4$VO$_3$	200	100	100
		2000	53	72
Zn(II)	Zn(NO$_3$)$_2$	100	69	-
		1000	71	87

Preliminary studies were carried out to investigate the best concentration of L-cysteine to use in order to reduce interferences in the determination of germanium. The enhancement of the germanium signal reaches a maximum when the concentration of L-cysteine is 0.02 % m/V. At this concentration, interference from a copper solution containing 500 μg ml^{-1} was also eliminated. For nickel, however, the interference from a solution containing 5 μg ml^{-1} nickel required a minimum concentration of 0.2% m/V L-cysteine. Signals from germanium were reduced at a L-cysteine concentration of 1.0 % m/V. Thus the concentration of L-cysteine chosen for interference reduction was 0.4 % m/V.

It is clear that both solid L-cystine and 0.4 % m/V L-cysteine reduce interferences substantially and to a similar degree in most cases. Thus concentrations of interfering ions can be tolerated at 100 to 1000 times the levels without the suppressant. Palladium strongly interfered in the production of SnH_4 and GeH_4 with both interference reducing agents and this was the only case where addition of solid L-cystine to the Pd(II) containing solution actually caused a reduction in the signal from germanium. With a solution of L-cysteine, however, the interference is reduced and solutions containing up to 10 μg ml^{-1} of palladium can be tolerated without a substantial loss in the signal from germanium.

Table 2 below shows the recoveries of germanium in the presence of various transition elements. Generally there is a very satisfactory recovery of germanium at concentrations of transition elements that would be encountered in the course of analysis. The sensitivity of the method is generally such that dilution of the solution will bring the concentration of the interfering element within the range where L-cysteine will reduce its interference to a negligible level.

Table 2

Interference reduction studies on the determination of germanium

Metal Ion Added	Amount Added	Germanium Recovery (%)		
		No interference-reducing agent (5 ml 1.0 ppb Ge)	L-cysteine 0.4% m/V (9.0 ml 0.5 ppb Ge)	L-cystine 0.4 g (5.0 ml 1.0 ppb Ge)
Ni(II)	1.0 ml 0.10 ppm	56	-	100
	2.0 ml 0.10 ppm	50	-	-
	1.0 ml 1.00 ppm	0	100	100
	1.0 ml 10.0 ppm	-	100	-
	2.0 ml 10.0 ppm	-	90	-
	1.0 ml 50.0 ppm	-	71	-
	1.0 ml 100 ppm	-	51	-
	1.0 ml 500 ppm	-	15	-
Cu(II)	1.0 ml 1.00 ppm	97	100	100
	1.0 ml 10.0 ppm	49	100	96
	1.0 ml 100 ppm	13	100	97
	1.0 ml 500 ppm	0	98	97
	1.0 ml 1000 ppm	-	100	-
	2.0 ml 1000 ppm	-	83	-
	1.0 ml 5000 ppm	-	72	-
	2.0 ml 5000 ppm	-	54	-
Co(II)	1.0 ml 1.00 ppm	100	98	100
	1.0 ml 10.0 ppm	34	100	100
	1.0 ml 50.0 ppm	-	103	-
	1.0 ml 100 ppm	0	91	92
	1.0 ml 500 ppm	-	73	-
	1.0 ml 1000 ppm	-	53	-
Fe(III)	1.0 ml 10.0 ppm	95	100	100
	1.0 ml 100 ppm	83	100	-
	1.0 ml 500 ppm	-	100	-
	1.0 ml 1000 ppm	28	97	96
	2.0 ml 1000 ppm	-	77	-
Zn (II)	0.5 ml 100 ppm	92	100	-
	1.0 ml 100 ppm	74	94	100
	0.5 ml 1000 ppm	-	100	-
	1.0 ml 1000 ppm	-	93	-
Cd(II)	0.5 ml 100 ppm	-	100	-
	1.0 ml 100 ppm	87	86	100
	0.5 ml 1000 ppm	-	73	-
	1.0 ml 1000 ppm	56	-	70

Hg(II)	0.5 ml	10.0 ppm	-	96	-
	1.0 ml	10.0 ppm	95	-	100
	0.5 ml	100 ppm	-	100	-
	1.0 ml	50 ppm	93	-	-
	1.0 ml	100 ppm	103	-	98
	0.5 ml	1000 ppm	-	100	-
	1.0 ml	1000 ppm	41	103	87
Pt(IV)	1.0 ml	0.10 ppm	-	100	-
	1.0 ml	1.00 ppm	-	99	-
	1.0 ml	10.0 ppm	-	96	-
	2.0 ml	10.0 ppm	-	94	-
	1.0 ml	100 ppm	-	96	-
	2.0 ml	100 ppm	-	94	-
Au(III)	1.0 ml	0.10 ppm	-	98	-
	1.0 ml	1.00 ppm	-	99	-
	1.0 ml	10.0 ppm	-	100	-
	0.5 ml	100 ppm	-	100	-
	1.0 ml	100 ppm	-	89	-
	2.0 ml	100 ppm	-	78	-
Pd(II)	1.0 ml	0.10 ppm	-	100	-
	1.0 ml	1.00 ppm	-	100	-
	0.5 ml	10.0 ppm	-	100	-
	1.0 ml	10.0 ppm	-	94	-
	1.0 ml	100 ppm	-	83	-
	2.0 ml	100 ppm	-	25	-
Mo(VI)	0.5 ml	100 ppm	-	100	-
	1.0 ml	100 ppm	100	-	100
	0.5 ml	1000 ppm	-	100	-
	1.0 ml	1000 ppm	78	100	96
	0.5 ml	10000 ppm	-	50	-
	1.0 ml	10000 ppm	0	-	20
V (V)	0.5 ml	200 ppm	-	106	-
	1.0 ml	200 ppm	97	-	100
	0.5 ml	2000 ppm	-	97	-
	1.0 ml	2000 ppm	98	-	97
Mn(II)	0.5 ml	1000 ppm	-	97	-
	1.0 ml	1000 ppm	94	-	97
	0.5 ml	10000 ppm	-	100	-
	1.0 ml	10000 ppm	86	-	93
Tl(I)	0.5 ml	100 ppm	-	100	-
	0.5 ml	1000 ppm	-	100	-
	1.0 ml	1000 ppm	-	94	-

Cr (VI)	0.5 ml	100 ppm	-	100	-
	1.0 ml	100 ppm	102	-	100
	0.5 ml	1000 ppm	-	97	-
	1.0 ml	1000 ppm	92	-	94
	0.5 ml	10000 ppm	-	72	-
	1.0 ml	10000 ppm	0	-	40
Ag	0.5 ml	22.6 ppm	-	94	-
	1.0 ml	22.6 ppm	92	91	100

Table 3 shows that the behaviour of hydride forming elements is different from the transition elements. It appears that there is relatively little interference from these elements under the conditions of the hydride forming reaction, except for selenium. In fact, L-cysteine appears to inhibit the formation of germane at high concentrations of selenium. Surprisingly, tin shows essentially no interference, even though the conditions for the generation of stannane are essentially the same as for the generation of germane.

Table 3
Recoveries of Germanium from Solutions Containing Other Hydride-Forming Elements

Metal Ion Added	Amount Added	Germanium Recovery (%)	
		9.0 ml 1.00 ppb Ge in 0.04 M HNO_3 + 1.5 ml 6% m/V $NaBH_4$	9.0 ml 0.50 ppb Ge in 0.04 M HNO_3 and 0.4 % m/V L-cysteine + 2.0 ml 6% m/V $NaBH_4$
Sn(II)	0.5 ml 100 ppm	95	97
	1.0 ml 100 ppm	93	94
	0.5 ml 1000 ppm	98	103
	1.0 ml 1000 ppm	108	97
Pb(II)	1.0 ml 100 ppm	97	100
	0.5 ml 1000 ppm	-	100
	1.0 ml 1000 ppm	94	93
As(III)	0.5 ml 100 ppm	88	103
	1.0 ml 100 ppm	85	97
Sb(III)	0.5 ml 100 ppm	100	100
	1.0 ml 100 ppm	88	94

Se(IV)	0.5 ml	10 ppm	85	100
	1.0 ml	10 ppm	83	100
	0.5 ml	100 ppm	88	69
	1.0 ml	100 ppm	95	39
	0.5 ml	1000 ppm	48	26
	1.0 ml	1000 ppm	25	10
Te(IV)	0.5 ml	100 ppm	98	100
	1.0 ml	100 ppm	98	94

Determination of Tin in NBS Standard Reference Material Benchmark Copper, Open Hearth Iron and Low Alloy Steel

To demonstrate the utility and accuracy of the present method of sample analysis, this method was applied to the determination of tin in the NBS Standard Reference Copper Benchmark Standards II and III, Open Hearth Iron 55E and Low Alloy Steel 363. Although no systematic interferences were anticipated on the basis of previous experiments, the method of standard additions was used to improve the accuracy of the determination and to see if there were any systematic errors associated with the determination. Three replicate, standard addition determinations were made for each standard, except for the Low Alloy Steel 363. The results are shown in Table 4. Paired t-tests[19] showed that there were no significant differences between the results obtained by the present method and the certified values.

Table 4
Concentrations of Tin in NBS Standard
Reference Materials ($\mu g\ g^{-1}$)

Sample	This work mean ± s.d. (n) from standard addition curve (20); n = number of points on the line	Mean ±Pooled s.d.	Certified Value
Benchmark Copper II	1.4 ± 0.1 (7) 1.5 ± 0.3 (5) 1.6 ± 0.1 (4)	1.5 ± 0.3	1.5

Benchmark Copper III	0.82 ± 0.05 (6) 0.76 ± 0.03 (5) 0.76 ± 0.11 (6)	0.78 ± 0.07	0.8
Open Hearth Iron 55E	69 ± 2 (7) 68 ± 1 (7) 68 ± 3 (7)	68 ± 2	70
Low Alloy Steel 363	$9.7 \pm (0.4) \times 10^2$ (5)	$9.7 \pm (0.4) \times 10^2$	$1.0_4 \times 10^3$

The slopes of the calibration curve and standard addition curve were parallel and the correlation coefficient factors of these regression lines were better than 0.99. The intercepts and standard deviations were calculated from the standard addition line by the method outlined by Miller and Miller[20] and adapted for use on the EXCEL database program.

Determination of Germanium in Copper and Iron

Determination of germanium in the "Benchmark" Copper I by the standard addition method (5 points) revealed that the germanium concentration was below the detection limit which, at the dilution employed in this determination, was $0.07 \ \mu g \ g^{-1}$. The sample spiked with $0.5 \ \mu g$ of germanium was determined to have a concentration of $0.52 \pm 0.02 \ \mu g \ g^{-1}$ by standard additions (5 points). This determination is consistent with a germanium concentration of less than $0.07 \ \mu g \ g^{-1}$.

The germanium concentration of the Open Hearth Iron 55E, determined by standard addition (5 points), was $18.3 \pm 0.7 \ \mu g \ g^{-1}$ and from a calibration curve (5 points) was $18.6 \pm 0.6 \ \mu g \ g^{-1}$. The sample spiked with $20 \mu g$ of germanium gave a recovery of 103% based on a 5 point standard addition determination. Excellent spike recoveries and the lack of systematic interferences, as revealed in the standard addition experiments, suggest that this method may be very useful in the

determination of germanium in standard reference materials. At the present time, no standard reference materials available from the National Bureau of Standards have certified values for germanium.

Detection Limit

The detection limit, defined as three times the noise, was found to be 20 pg ml^{-1} (or 100 pg for a 5 ml sample)for tin and, with a 9.0 ml sample, the detection limit for the determination of germanium is 20 pg ml^{-1} (or 180 pg for a 9.0 ml sample).

Conclusion

A solution of 0.4% m/V L-cysteine reduces interferences from transition elements to a point where the determination of tin and germanium in most matrices can be made rapidly. This method shows promise as a method for the determination of germanium in environmental and metallurgical applications.

Ackowledgements

References

1. Robbins, W. B. and Caruso, J. A., *Anal. Chem.*, 1979, **51**, 889A.
2. Hahn, M. H., Wolnik, K. A., Fricke, F. L., Caruso, J. A., *Anal. Chem.* 1982, **54**, 1048.
3. Nakahara, T., *Prog. Anal. At. Spectrosc.*, 1983, **6**, 163.
4. Browner, R. F. and Boorn, A. W., *Anal. Chem.*, 1984, **56**, 875A.
5. Pollock, E. N. and West, S. J., *At. Abs. Newsl.*, 1973, **12**, 6.
6. Andreae, M. O., *in* Grasshoff,K., Erhardt, M., and Kremling, K., *Editors*, "Methods of Seawater Analysis" Second Edition, Verlag Chemie, Weinheim, 1983, p.230.
7. Brindle, I. D. and Ceccarelli-Ponzoni, C. M., Analyst (London), 1987, **112**, 1547.
8. Vijan, P. N. and Chan, C. Y., *Anal. Chem.*, 1976, **48**, 1788.
9. Fleming, H. D. and Ide, R. G., *Anal. Chim. Acta*, 1976, **83**, 67.
10. Thompson, M. and Pahlavanpour, B., *Anal. Chim. Acta*, 1979, **109**, 251-258.
11. Nakahara, T., *Appl. Spectrosc.*, 1983, **37**, 539.
12. Jin, K. Terada, H., and Taga, M., *Bull Chem. Soc. Jpn.*, 1981, **51**, 2934.
13. Boampong, C., Brindle, I. D., Le, X. C., Pidwerbesky, L., Ceccarelli Ponzoni, C. M., *Anal. Chem.*, 1988, **60**, 1185.
14. Boampong, C., Brindle, I. D., Ceccarelli Ponzoni, C.M., *J. Anal. Atom. Spectrom.*, 1987, **2**, 197.
15. Hodge, V.F., Seidel, S.L., Goldberg, E.D., *Anal. Chem.*, 1979, **51**, 1256.
16. Brindle, I.D. and Le, X.-c., *Analyst*, 1988, **113**, 1377.
17. Ebdon, L., Norman, P., and Sparkes, S.T., *Spectrochim. Acta*, 1987, **42B**, 619.
18. Tsujii, K. and Kuga, K., *Anal. Chim. Acta*, 1978, **101**, 199.
19. Miller, J. C. and Miller, J. N., *Statistics for Analytical Chemistry*; John Wiley and Sons: New York City, 1984; pp 56.
20. ibid. pp. 82.

USE OF THE HOT GAS SLURRY TECHNIQUE FOR SOLID SAMPLE INTRODUCTION FOR ICP-AES.

Lyne Gervais[*], Eric D. Salin, Department of Chemistry, McGill University, 801 Sherbrooke St. W., Montreal, Quebec, Canada H3A 2K6

INTRODUCTION

One of the primary limitations of conventional laboratory instrumentation is its inability to do very low level determinations directly in solid materials. Recent experience in our laboratory with "real solid samples" has led us to investigate slurry sample introduction methods for ICP-AES. Slurry methods have the distinct advantage that it produces a static signal allowing sufficient time for background correction methods and even rapid scanning spectrometers to be used. Analysis by the slurry methods are simple to perform and require very limited modification to the ICP system normally used for liquids. Our initial experiments demonstrated to us that slurries did offer many of the claimed advantages, e.g. calibration with aqueous standards, however detection limits were significantly reduced. This is to be expected since a 1% slurry is, in essence, a 1:100 dilution of the sample. Higher percentage slurries could be used but handling of the suspended solid becomes more difficult as the load increases.

In our search for better detection limits with slurries we have followed a general methodology used by Veillon and Margoshes[1]. The use of a heated spray chamber followed by a condenser was reported to result in a dry aerosol. Configurations of this type are now common with ultrasonic nebulisers where

---

Veillon, C.; Margoshes, M. *Spectrochim. Acta* **1968**, 23B, 553-555.

the very large amount of solvent must be removed before reaching the atomisation source. This papers reports on the use of this general design with a Babington type nebuliser for the introduction of slurry. The key addition to the system is the use of a hot gas through the nebuliser. This new system was expected to provide a sufficient input of thermal energy through the nebuliser gas and the walls of the spray chamber for the development of either smaller droplets or possibly aerosol particles which should transport to the plasma with much higher efficiency. Higher transport efficiency should result in improved detection limits if plasma excitation conditions remain essentially unchanged.

EXPERIMENTAL

HOT NEBULISER GAS SLURRY SYSTEM

A Legere nebuliser[2] and spray chamber were used with a cooled inner coil condenser similar to the arrangement of Veillon and Margoshes[1]. The normal PVC sample input tube was replaced by a stainless steel tube to allow the use of a high temperature environment. The sample is fed to the nebuliser by a peristaltic pump. The argon gas was heated to the desired temperature when passed through copper tubing wrapped with a heating tape. The temperature of the gas was adjusted with an electronic temperature controller which receives feedback from a thermocouple placed in the copper gas line immediately before the nebuliser body. The glass spray chamber was wrapped with a heating tape which extended from the nebuliser to over the glass connector leading to the condenser. A variable power supply was used to control heating of the spray chamber while the surface temperature was

---

Legere, G.; Burgener, P. *ICP Info.Newsl.* **1985**, 11(7), 447-456.

measured with a thermocouple. The cooling water for the condenser was maintained at 10 C (+/- 2 C) by circulation in an ice-water bath. The inner coil condenser was operated in a vertical position. The spray chamber is at an 8 degree angle with the horizon to allow free flow to the drain. In this work, the heated spray chamber was used to maintain the hot environment produced by the hot nebuliser gas along the path of the aerosol to the condenser.

SAMPLE PREPARATION

The composition of the aqueous sample was of 20 ppm in aluminum, cadmium, copper, lead, nickel and zinc. Analytical grade salts or spectroscopic grade powders were used for the preparation of those solutions. The marine sediment reference material BCSS-1 (National Research Council of Canada) was used for the slurry sample. BCSS-1 is delivered in a format which passes No 120 (125 micron) screen. Particles of less than 3 microns (97 % of the particles) were obtained with a thirty minutes grinding in an agate vial. The 0.5% (w/v) BCSS-1 suspension in water was treated with a 30 minutes sonication using an ultrasonic bath and then magnetically stirred during the analysis to keep the sample uniformly suspended. All samples were made with deionized doubly-distilled water.

PROCEDURE

A Thermo Jarrell-Ash ICAP 61 direct reading spectrometer was used for all measurements with conventional operating conditions. Intensity measurements were obtained for a water blank, the multielement aqueous solution and the 0.5% BCSS-1 slurry. The signals used were the average of 10 repeats of a 10 seconds integration. For both types of samples, aluminum, cadmium, copper, nickel, lead and zinc were monitored. With the exception of

aluminum, all are part of the trace constituent list for BCSS-1, our target "real sample".

An optimisation of the hot nebuliser gas sample introduction system was done for two factors: (1) the temperature of the nebuliser gas and (2) the temperature of the spray chamber. The gas temperature effect was studied over the range of 23 C to 235 C. Spray chamber temperatures ranged from 23 C to 250 C for the liquids and up to 185 C for the slurry.

RESULTS AND DISCUSSION

The Hot Nebuliser Gas Slurry system was first tested and optimised for liquid samples. An optimum Es/Blk (Emission signal to blank) was found at a gas temperature of 205 C and a spray chamber temperature of 110 C. The improvement of the optimisation factor at a specific operating condition was calculated with respect to the optimisation factor found when using the same system at room temperature. For those liquid analysis conditions, an improvement of 4.6 of the Es/Blk was obtained.

The optimisation for slurries showed slightly different optimum conditions than those for liquids. An input gas temperature of 235 C instead of 205 C with the same spray chamber temperature (110 C) was the most efficient. Under these conditions, an Es/Blk improvement of 6.5 was obtained. Running the BCSS-1 slurry under the liquid sample optimum conditions, an improvement of 4.9 was observed.

The use of the hot nebuliser gas system for the nebulisation of either liquids or suspensions results in a significant improvement of the emission signal-to-blank-ratio observed. Therefore the analytical performance of the ICP-AES system, particularly the detection limit, could be improved by this sample

introduction system. In its current state, a short term instability of the signal is observed which results in poor noise levels. Work is in progress to solve this problem.

At this point we have optimised the temperatures of the spray chamber, gas heating system and the viewing height for one set of conditions. To gain a more correct estimate of the power of the system, a global optimisation would be necessary. Effect of the operating conditions such as nebuliser gas flow, nebuliser liquid flow, plasma power and gas flows and condenser would have to be studied. While we are encouraged by the improvement of 6.5, we expect that further improvement should be possible when these other factors are included in the optimisation process.

D16

ADVANCED TECHNOLOGY FOR DESTRUCTION OF WATERBORNE ORGANIC POLLUTANTS

Paul Skala and Harry Whittaker
Engineering Section, Environmental Emergencies Technology Division,
River Road Environmental Technology Centre, Ottawa, Ont. K1A 0H3.

and

Hussain Al-Ekabi[1], Mike Robertson, Ali Safarzadeh-Amiri and
Bob Henderson
Nulite, A Division of Nutech Energy Systems Inc., 317 Consortium
Court, London, Ontario N6E 2S8.
Phone (519)668-7530   Fax (519)685-9800

ABSTRACT

Nulite is developing an advanced technology for water treatment. This technology uses TiO_2 photocatalytic mineralization of organic pollutants into innocuous inorganic species. A prototype photoreactor consisting of a near UV lamp surrounded coaxially by a fibreglass mesh to which a thin layer of TiO_2 (anatase) is firmly bonded was fabricated by Nulite. The Nulite reactor was tested on each individual as well as an equimolar mixture of all components of the Gloucestor Landfill[2] water and found to be very efficient. The reactor results indicate that this technology will have wide application in the cleanup of organically contaminated waters since Gloucestor water contains four different classes of the major concern organic pollutants.

---

[1]correspondence should be addressed to this author

[2]Gloucester Landfill is a landfill site situated in the Township of Gloucester outside of Ottawa, Ontario. From 1968 – 1984, it has been the repository for chemical wastes from local laboratories.

INTRODUCTION

The presence of chlorinated hydrocarbons and other organic pollutants in natural and drinking waters has sparked much concern both in Europe and North America. While many of these pollutants are nonbiodegradable and persistent in the environment, some can be degraded by microbiological processes. However, microbial degradation requires a long period of time. In contrast, other methods are generally effective in removing organic pollutants from water, but most of them merely transfer the pollutants from a large volume of contaminated material into a smaller volume of concentrate which then requires expensive treatment or special dumping permits. Activated carbon treatment has been widely used to remove most organic compounds from such large volumes. However, the disposal of the spent carbon poses a severe problem as landfill sites come under increasing regulatory restrictions. Thus, an exhaustive search is currently underway for an alternative treatment of these concentrates and the original waters.

The photocatalytic mineralization of chlorinated hydrocarbons and other organic pollutants in water mediated by illuminated TiO_2 has been demonstrated[1-8]. This approach represents a highly promising complete treatment since it would render all organic pollutants virtually harmless.

The illumination of TiO_2 with light of wavelengths <400nm produces photoelectrons in the conduction band and positive holes in the valence band:

$$TiO_2 \ \text{-------}\text{>} \quad e^-_{CB} + h^+_{VB} \tag{1}$$

The hole(h^+_{VB}) and electron (e^-_{CB}) are both able to participate in chemical reactions. At the surface, the hole reacts with either adsorbed H_2O or with a surface hydroxyl group to form an hydroxyl radical which is a powerful oxidizing agent for organic pollutants.

$$h^+_{VB} + H_2O \ (ads.) \ \text{-------}> \quad OH^{\cdot} \ + \ H^+ \quad\quad (2)$$

$$h^+_{VB} + OH^- \ (sur.) \ \text{-------}> OH^{\cdot} \quad\quad (3)$$

Simultaneously, the molecular oxygen adsorbed on the surface will react with the electron to form superoxide ion ($O_2^{\cdot -}$).

$$e^-_{CB} + O_2 \ \text{-------}> \quad O_2^{\cdot -} \quad\quad (4)$$
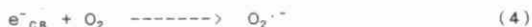
This process delays the electron/hole recombination and thus makes the degradation of the organic pollutants more efficient.

The TiO_2 photocatalyst, in most of the earlier studies on the photocatalytic mineralization of organic pollutants, was freely suspended in aqueous solutions and thus filtration and resuspension of the photocatalyst are obvious practical problems. This prompted Nulite to build a prototype photoreactor in which the TiO_2 (anatase) is firmly supported on a fibreglass mesh. This photo-reactor could be used for both single-pass and multi-pass mode experiments.

The earlier studies on the TiO_2-mediated degradation of organic pollutants have dealt with the degradation of single organic compounds. It was, therefore, desirable to test the performance of the Nulite prototype reactor not only on a variety of single organic compounds but also on an equimolar mixture of Gloucestor water components.

173

EXPERIMENTAL

The Nulite Prototype Photoreactor

The Nulite prototype photoreactor comprises a jacket, a lamp and a photocatalytic sleeve. The lamp emits ultraviolet light in the 300-400 nm range and is mounted coaxially within the jacket. Around the lamp lies a sleeve formed of fibreglass mesh which is coated with a firmly bonded layer of titanium dioxide (anatase).

The anatase layer is activated by ultraviolet light. Contaminated water flows through the reactor in parallel with the lamp. As the water passes through the sleeve, the open pore configuration of the mesh creates trubulant mixing. In concert with the large surface area of the mesh, this mixing ensures contact between the organic pollutant and the photocatalyst.

The reactor was placed vertically and connected to a 4000 ml glass reservoir and a peristaltic pump. The flow of the recirculated solution through the system was controlled by changing the electrical input of the pump with a rheostat, and measured with a flow meter. All tubing used in connecting the apparatus was leach resistant teflon or viton.

General Procedure

The Nulite prototype photoreactor was operated at constant temperature (27°C) by placing the reservoir into a suitable water bath. Typically, a standard volume (3000ml) of a solution of the desired contaminant of known concentration (5×10^{-5}M; 3.5-5ppm) was made up using HPLC grade water and good grade chemicals. This solution except for a small aliquot (about 5ml), was placed in the reservoir and pumped through the system, which had been previously washed with HPLC grade water and drained. As soon as the desired

flow rate and temperature were established a sample of the solution was taken, usually at the reactor inlet, and the UV light was turned on. Samples were then taken at regular intervals and analyzed by GC, while the solution was pumped through the system at controlled flow rate and temperature. The small aliquot, taken before the solution was transferred to the reservoir was analyzed and used as a standard to calibrate the GC detector's response to a given compound(s). The GC analyses of all samples were performed on a Hewlett Packard 5830A instrument, using a Megabore DB 1701 column (30m long, 0.4mm ID), flame ionization detector at 260°C, and direct injection of 1-2 μl of sample. The injection port temperature was held at 260°C and helium (1.5ml/min) was used as carrier gas. Oven temperature varied depending on the particular needs of each compound or mixture, typically 150°C for phenol analysis, 40-80°C for most other organics. For the analysis of the equimolar mixture, the following temperature program was used: 1.5 min. initial hold at 20°C; 7°C/min (5 min); 15°C/min (7 min); 30°C/min until 210°C was attained.

RESULTS AND DISCUSSION

Degradation of Phenol

Initially, it was desirable to test the Nulite prototype reactor with a known TiO_2 photocatalyzed mineralization reaction. The mineralization of phenol was chosen for a number of reasons: (1) earlier studies suggested that complete mineralization of phenol mediated by illuminated TiO_2 is fairly fast, (2) being an aromatic compound, it represents one of the main classes of compounds for which the Nulite reactor was designed and (3) good solubility in water and low volatility allow easier sample manipulation and higher accuracy of kinetic measurements.

It is important, in a flow solid-liquid reaction, to determine the extent to which mass transfer may affect the overall rate of mineralization in the Nulite reactor and to establish a kinetic region in which the mass transfer is sufficiently rapid to have no noticeable effect on the rate of the overall degradation process. Thus, the degradation of phenol (5 ppm) in aerated aqueous solutions vs irradiation time was investigated at different flow rates. The results indicated that the flow rate has little effect on the overall rate of phenol degradation between 0.05 and 0.25 gpm but has no effect between 0.25 and 0.5 gpm where the rate of degradation seems to be constant within the limits of the accuracy of the GC analysis. Therefore, the limit of the kinetic region is found to be around 0.25 - 0.5 gpm for the degradation of 5 ppm of phenol. Since other organic compounds may, in principle, be mineralized faster than phenol, the flow rate of 0.5 gpm was used for all subsequent kinetic measurements.

Figure 1 illustrates the degradation of phenol (5 ppm) in aerated aqueous solution as a function of irradiation time. The degradation proceeds very efficiently. Plots of $\ln(C_o/C)$ vs irradiation time shows good linearity indicating that the reaction approximates first order kinetics to a high degree of degradation ($>80\%$). The apparent rate constant is found to be 0.067 min^{-1}. This behaviour could be rationalized in terms of a modified form of the Langmuir-Hinshelwood (L-H) kinetic treatments which has been used successfully to describe many solid-liquid reactions[9].
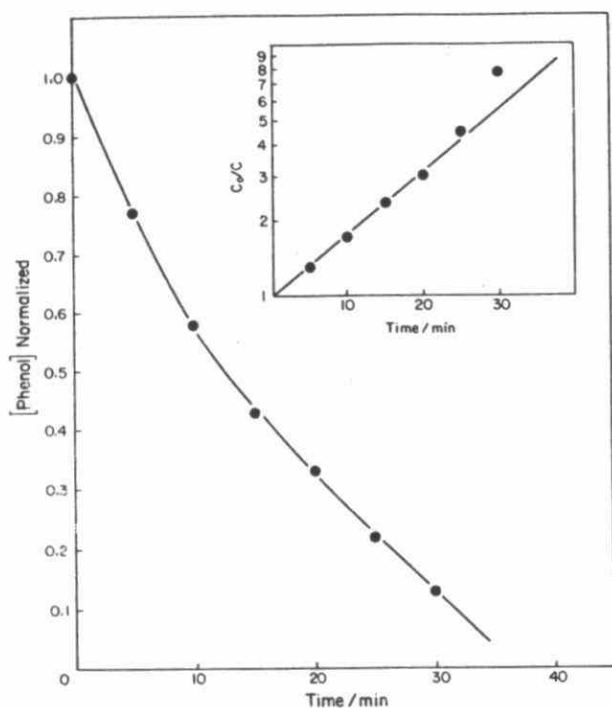
Figure 1. Plot showing changes in the concentrations of phenol as a function of irradiation time. The insert is a plot of $\ln(C_0/C)$ vs time.

According to the L-H kinetic treatment, a unimolecular surface reaction, where the reactant is significantly more strongly adsorbed than the product, will obey an equation of the form:

$$\text{Rate} = -\,dC/dt = k_r\,\theta = k_r[KC_o/(1+KC_o+K_wC_w)] \qquad (5)$$

where k_r is the reaction rate constant, θ, is the fraction of the surface covered by the reactant (contaminant), C_o is the initial concentration of the organic reactant in solution, K is the adsorption coefficient of the reactant, K_w is the adsorption coefficient of water and C_w is the concentration of water (55.5M).

Similarily, a bimolecular reaction between two surface species (without significant mutual displacement) obeys equation (6)

$$\text{Rate} = k_r\,\theta_1\,\theta_2 \qquad (6)$$

where θ_1 and θ_2 are the fractions of the surface covered by the surface reactant species. Equation (6) reduces to a form similar to that of equation (5) where the concentration of one of the species remains constant. Since the concentration of the reactive species (OH radical) remains constant (the light intensity and the amounts of TiO_2 and water are kept constant), equation (5) can be adapted to explain our results.

Since the concentration of water C_w is much greater than the solute C_o, and C_w remains essentially constant, the part of TiO_2 surface covered by the solvent (water) is approximately constant at all the reactant concentrations used.

Integration of equation (5) gives equation (7).

$$\ln(C_o/C) + (K/1+K_wC_w)(C_o-C) = (k_rK/1+K_wC_w)t \qquad (7)$$

Obviously, equation (7) is the sum of zero-order and first-order rate equations and their contribution to the overall reaction depends essentially on the initial concentrations C_o. When C_o is very low, equation (7) reduces to equation (8) which is a first-order rate equation.

$$\ln(C_o/C) = k't \qquad (8)$$

where k' is the apparent first-order rate constant. Equation (8) explains the results presented in this paper.

Degradation of the Gloucestor Water Components

The main objective of this study was not only to test the performance of the Nulite prototype reactor on one particular sample of contaminated water, but also to compare rates of degradation of various compounds with different functional groups and possibly relate the rates of degradation of each compound to its structure.

GC analysis of the Gloucestor water sample revealed 12 main organic contaminants (Table 1). Therefore, it was considered important in this context to determine the reactor's potential preference for some classes of compounds. The sample of Gloucestor landfill water contains at least one compound of each of the main classes that one considered the most likely targets of an industrial application of the Nulite reactor.

179

Table 1

Organic contaminants in Gloucestor ground water and RO concentrate.

COMPOUNDS	FEED/ppm	CONCENTRATE/ppm
Benzene	1.177	9.570
Toluene	0.204	1.528
Chlorobenzene	0.008	0.025
1,1-Dichloroethylene	0.010	0.050
1,2-Dichloroethylene	-----	-----
Trichlorethylene	0.417	2.091
Diethylether	0.273	1.986
Tetrahydrofuran	-----	-----
1,1-Dichloroethane	0.126	0.792
1,2-Dichloroethane	0.128	0.748
1,1,1-Trichloroethane	0.145	1.117
Chloroform	0.338	2.313

For this reason, it provided an ideal model for the first trial of the reactor. Also, the knowledge of the reactor's selectivity towards all components of this mixture is likely to become a valuable tool in later predictions of degradation rates of related compounds. Thus, first it was important to investigate the individual degradation of all compounds involved plus tetrahydrofuran which was included at this stage in order to provide more reference data on the degradation of ethers. The next stage was the treatment of an equimolar mixture of all compounds except tetrahydrofuran which interfered with the GC analysis of 1,1,1-trichloroethane.

To simplify the discussion, the Gloucestor water components are divided into four different classes of pollutants. They are: (1) aromatic compounds, (2) chlorinated ethylenes, (3) ethers and (4) chlorinated alkanes. The degradation data of the components of each class of pollutants are presented separately.

Degradation of the Aromatic Compounds of the Gloucestor Water

The individual degradation of aerated aqueous solutions of benzene, toluene and chlorobenzene was investigated using the Nulite reactor and found to be very efficient. Figure 2 shows the degradation of toluene as a function of irradiation time. The reaction follows first-order kinetics as a straight line was obtained when $\ln(C_o/C)$ was plotted vs irradiation time. The apparent rate constant was 0.072 min^{-1}. The degradation of benzene and chlorobenzene also follows first-order kinetics with apparent rate constants of 0.071 and 0.052 min^{-1}, respectively. Clearly, the degradation rate constants of toluene and benzene are higher than that of chlorobenzene. The OH radical is known to have an electrophilic character[10] and thus it would be, generally, expected that aromatic compounds substituted with an electron donating group are more readily attacked than those substituted with an electron withdrawing group such as Cl.

Degradation of the Chlorinated Ethylenes of the Gloucestor Water

The individual degradation of aerated aqueous solutions of 1,1-dichloroethylene, 1,2- dichloroethylene and trichloroethylene was investigated as a function of irradiation time. The degradation of all three compounds was very efficient. The data for
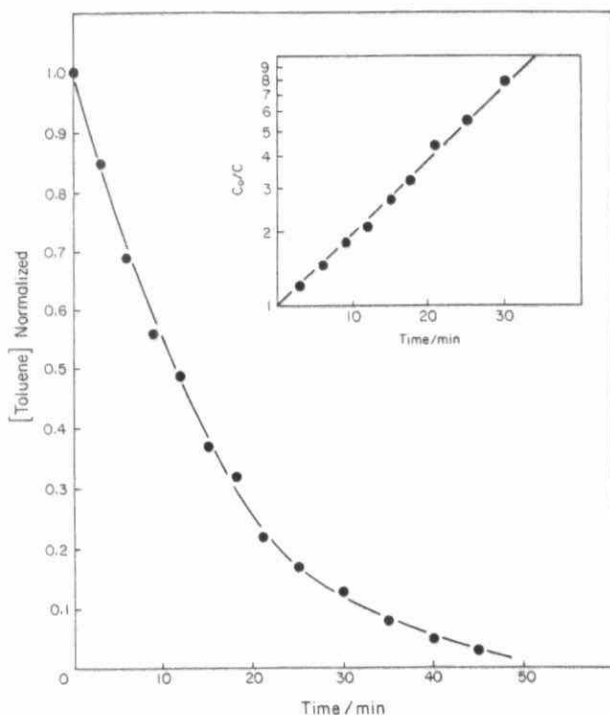
181

Figure 2. Plot showing changes in the concentrations of toluene as a function of irradiation time. The insert is a plot of $\ln(C_o/C)$ vs time.
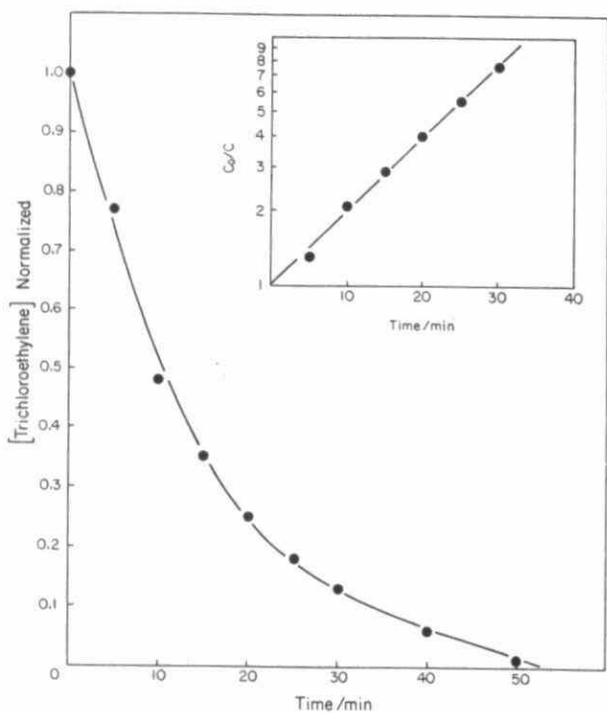
Figure 3. Plot showing changes in the concentrations of trichloro-
ethylene as a function of irradiation time. The insert is a plot
of $\ln(C_o/C)$ vs time.

trichloroethylene are shown in Figure 3. The reaction follows first-order kinetics with an apparent rate constant of 0.069 min^{-1}. The rate constants for degradation of 1,1-dichloroethylene and 1,2-dichloroethylene are 0.083 and 0.05 min^{-1}, respectively.

Degradation of the Ethers of the Gloucestor Water

Although tetrahydrofuran was not present in the Gloucestor water sample, it was included in this study for two reasons: (1) it had been previously found in other Gloucestor water samples taken from other sites and (2) it provides more reference data on degradation of ethers. Thus, the photocatalytic degradation of tetrahydrofuran and diethylether in aerated aqueous solutions was investigated. The degradation of both compounds was efficient and follows first-order kinetics with apparent rate constants of 0.048 and 0.052 min^{-1} for tetrahydrofuran and diethylether, respectively. Figure 4 demonstrates the degradation of diethylether as a function of irradiation time.

It is noteworthy that the degradation rates of tetrahydrofuran and diethylether are comparable with those of aromatic compounds and chlorinated ethylenes. Diethylether and tetrahydrofuran are known to be good hydrogen donating compounds and thus abstraction of their $\propto$ - hydrogen atoms by OH radical is expected to be fast. This would render the degradation high efficient.

Degradation of the Chlorinated Alkanes of the Gloucestor Water

Unlike the degradation of the aromatic compounds, the chlorinated ethylenes and the ethers, the degradation of 1,2-dichloroethane, chloroform and 1,1,1-trichlorethane under similar conditions was considerably slower. However, complete degradation could be achieved with longer irradiation time. The degradation
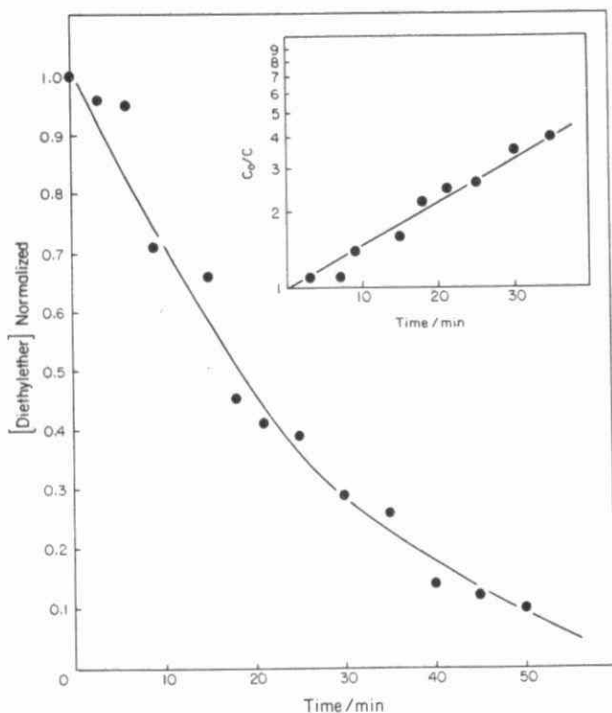
Figure 4. Plot showing changes in the concentrations of diethylet-
her as a function of irradiation time. The insert is a plot of
ln(C_o/C) vs time.

185

for all three cases follows first-order kinetics and the data for 1,2-dichlorethane are depicted in Figure 5. The degradation rate constants for chloroform, 1,2-dichloroethane and 1,1,1-trichloroethane were 0.01, 0.014 and 0.01 min^{-1}, respectively. Clearly, the rates of degradation of the chlorinated ethylenes are higher than those of the chlorinated alkanes. Alkanes are known to be less reactive towards OH radical attack than alkenes.

Degradation of an Equimolar Mixture of the Gloucestor Water Components

Having investigated individually the photocatalytic degradation of the Gloucestor water components, it was important to test the degradation of an equimolar mixture of the Gloucestor water components in Nulite's reactor. In this regard, it should be mentioned that most of the reports so far published on the TiO_2-mediated mineralization of organic pollutants have dealt with the degradation of single organic compounds. Thus, the degradation of an equimolar mixture (2.5×10^{-5}M; 1.75-2.5ppm each) of benzene, toluene, chlorobenzene, diethylether, 1,1-dichloroethylene, 1,2-dichloroethylene, trichloroethylene, 1,2-dichloroethane, chloroform and 1,1,1-trichloroethane in aerated aqueous solution was carried out as a function of irradiation time. In agreement with the individual degradation data, the results illustrated in Figure 6 clearly show that benzene, toluene, chlorobenzene, 1,1-dichloroethylene, 1,2-dichloroethylene, trichloroethylene and to a lesser extent, diethylether degrade with comparable rates. Approximately 100 min. was enough to degrade completely these components. By contrast, and in agreement with the individual degradation data, the degradation of chloroform, 1,2-dichloroethane and 1,1,1-trichloroethane was much slower. However, their degradation became faster once the other components had been degraded.
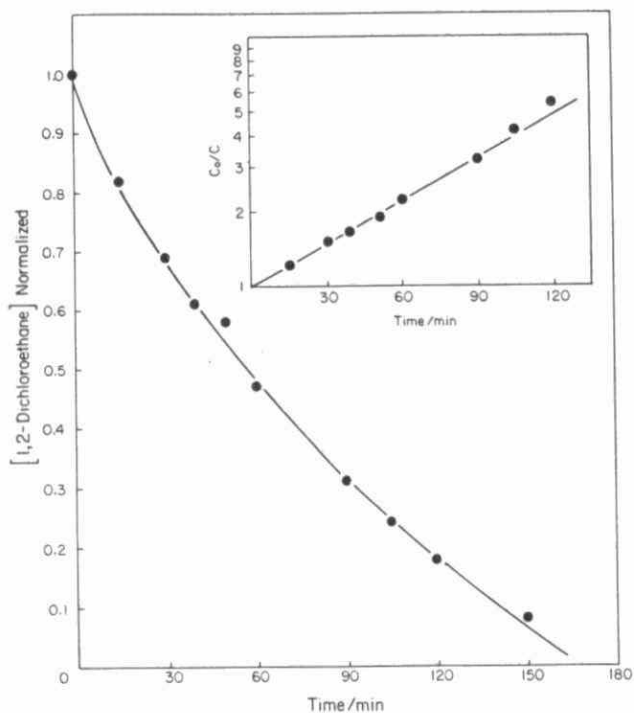
Figure 5. Plot showing changes in the concentrations of 1,2-dichloroethane as a function of irradiation time. The insert is a plot of ln(C_o/C) vs time.
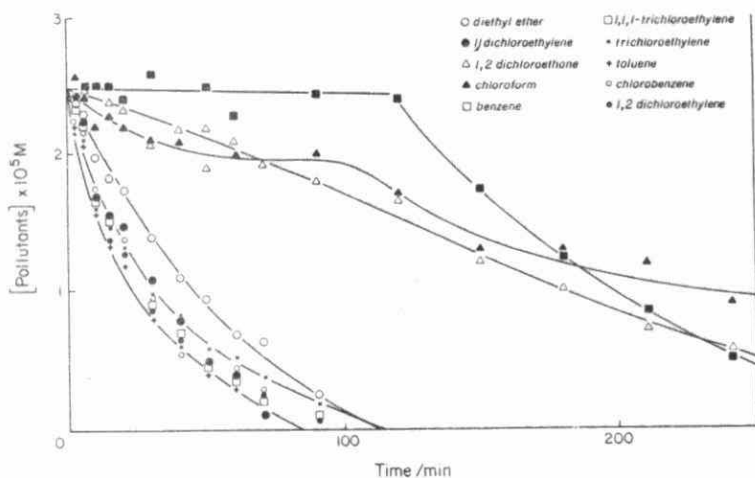
Figure 6. Plot showing changes in the concentrations of an equimoler mixture of Gloucestor water components as a function of irradiation time.

It is noteworthy that the degradation of all compounds in this equimolar mixture followed first-order kinetics. Representive plots of $\ln(C_o/C)$ <u>vs</u> time for benzene, 1,2-dichloroethylene and 1,2-dichloroethane are presented in Figure 7 and the apparent rate constants for all the degraded components are presented in Table 2 along with the individual degradation rate constants.

Table 2

Apparent first-order rate constants k' and $t_{1/2}$ for the degradation of Gloucestor water components in a continuous recirculation mode at 0.5 gpm flow rate.

| Compound	Individual		Mixture	
	$k' \times 10^3$, min^{-1}	$t_{1/2}$, min	$k' \times 10^3$, min^{-1}	$t_{1/2}$, min
Benzene	71	9.8	33	21.0
Toluene	72	9.6	37	18.7
Chlorobenzene	52	13.3	31	22.4
1,1-Dichloroethylene	83	8.4	29	23.9
1,2-Dichloroethylene	50	13.9	29	23.9
Trichloroethylene	69	10.0	30	23.1
Diethylether	52	13.3	20	34.7
Tetrahydrofuran	48	14.4	--	----
Chloroform	10	69.3	5	138.6
1,2-Dichloroethane	14	49.5	4	173.3
1,1,1-Trichloroethane	10	69.3	--	----
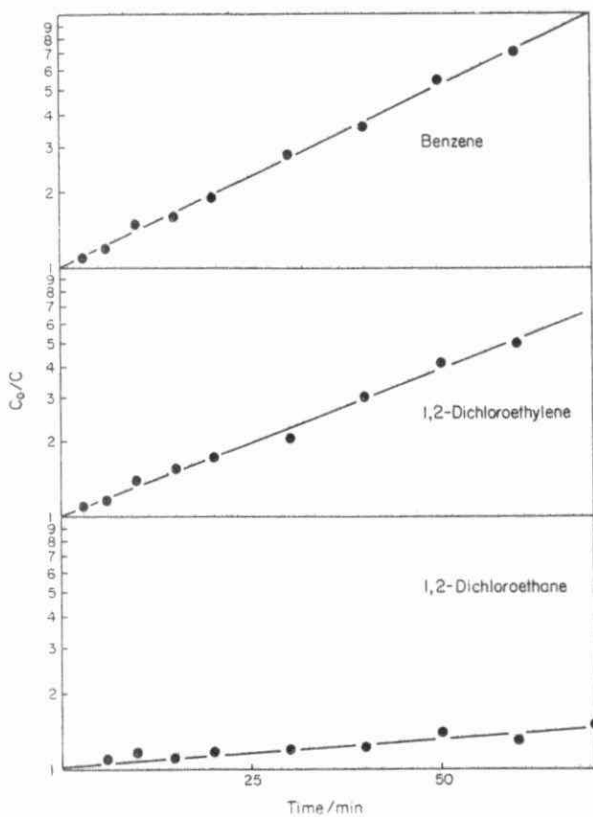
Figure 7. Plot of $\ln(C_0/C)$ vs time for benzene, 1,2-dichloroethyl-
ene and 1,2-dichloroethane of the Gloucestor water mixture.

CONCLUSIONS

1) The Nulite prototype photoreactor degraded with high efficiencies all components of the Gloucestor water both individually and in an equimolar mixture. Clearly, this is an advanced step in the degradation of organic pollutants via heterogeneous photocatalysis since all of the TiO_2-mediated degradation studies so far published have dealt only with the degradation of single organic compounds. The degradation of the Gloucestor water components by the Nulite reactor acquires special importance not only because of the large number of organic pollutants tested individually or in an equimolar mixture but also because of the diverse nature of the organic compounds involved. The Gloucestor water contains organic compounds belonging to different classes of organic pollutants which are of the greatest environmental concern today.

2) The Nulite reactor degraded the aromatic compounds, (both chlorinated and unchlorinated), the chlorinated ethylenes and diethylether of the Gloucestor water with high efficiencies while the rates of degradation of the chlorinated alkanes were considerably slower. Since virtually any treatment of a real mixture of contaminants is in fact a case of competitive degradation where different compounds (contaminants) compete with each other for the photocatalyst, the selectivity of the photocatalyst in such cases is an important factor. A reactor capable of oxidizing primarily the compounds of most concern, even in the presence of a high concentration of other compounds, would have a definite advantage over one that either did not discriminate or treated the most noxious substances last, with minimum efficiency, or not at all.

191

3)   Although the Nulite reactor showed remarkable degradation
     efficiencies towards Gloucestor water which contains four
     different classes of organic pollutants, it should be noted
     that the reactor is an early stage prototype and work is in
     progress to optimize its degradation capacity.

ACKNOWLEDGEMENTS

REFERENCES

1.   Carey, J.H.; Lawrence, J.; Tosine, H.M., <u>Bull. Environ.
     Contam. Toxicol.</u>,1976, <u>16</u>, 697.

2.   (a) Hsiao, C.-Y.; Lee, C.-L.; Ollis, D.F., <u>J.Catal</u>, 1983,
     82,418.   (b) Pruden, A.L.; Ollis, D.F., <u>Environ. Sci.
     Technol.</u>, 1983,<u>17</u>,628. (c) Pruden, A.L.; Ollis, D.F.,
     <u>J.Catal.</u>, 1983,<u>82</u>,404 (d) Ollis, D.F.; Hsiao, C.-Y.; Budiman,
     L.; Lee, C.L.,<u>J.Catal.</u>,1984,<u>88</u>,89.   (e) Nguyen, T.; Ollis,
     D.F.; <u>J.Phys. Chem.</u>,1984,<u>88</u>,3386. (f) Ollis, D.F., <u>Environ.
     Sci. Technol.</u>,1985,<u>19</u>,480. (g) Ollis, D.F., <u>J. Catal.</u>,
     1986,<u>97</u>,5693.

3.   (a) Matthews, R.W., <u>J.Chem. Soc.Faraday Trans. 1.</u> 1984,<u>80</u>,457.
     (b) Matthews, R.W., <u>J.Catal.</u>, 1986,<u>97</u>,565. (c) Matthews,
     R.W., <u>Water Res.</u>,1986,<u>20</u>,569. (d) Matthews, R.W., <u>J.Phys.
     Chem.</u>, 1987,<u>91</u>,3328.   (e) Matthews, R.W., <u>Solar Energy</u>,
     1987,<u>38</u>,405. (f) Matthews, R.W., <u>Aus. J. Chem.</u>, 1987,<u>40</u>,667

4. (a) Barbeni, M.,Pramauro, E.; pelizzetti, E.; Borgarello, E.; Gratzel, M.; Serpone, N., _Nouv. J. Chim.,_ 1984,$\underline{8}$,547. (b) Hidaka, H.; Kubota, H.; Gratzel,M.; Serpone, N.; Pellizzetti, E., _Nouv. J. Chim.,_ 1985,$\underline{9}$,67 (c) Hidaka, H.; Kubota, H.; Gratzel, M.; Delizzetti, E.; Serpone, N., _J. Photochem.,_ 1986,$\underline{35}$,219. (d) Pelizzetti, E.; Borgarello, M.; Minero, C.; Pramauro, E.; Serpone, N.,_Chemosphere,_ 1988,$\underline{17}$,499. (e) Barbeni, M.; Morello,M.; Pramauro, E.;Pelizzetti, E.; Vincenti, M.; Borgarello, E.; Serpone, N.,_Chemosphere,_ 1987,$\underline{16}$,1.165 (f) Al-Ekabi, H.; Serpone, N. _J.Phys. Chem.,_ 1988,$\underline{92}$,0000

5. (a) Okamoto. K.; Yamamoto, Y.; Tanaka,Ho; Tanaka,M., _Bull.Chem.Soc. Jpn.,_ 1985,$\underline{58}$,2015. (b) Okamoto,K.; Yamamoto,Y.; Tanaka,H.; Itaya, A., _Bull.Cnem.Soc.Jpn.,_ 1985, $\underline{58}$,2023.

6. (a) Kraeutler, B.; Bard, A.J., _J.Amer. Chem. Soc.,_ 1978. $\underline{100}$,5985. (b) Izumi, I.; Dunn. W.W.; Wilbourn, K.O.; Fan, F.R.F.; Bard, A.J., _J.Phys.Chem.,_ 1980,$\underline{84}$,3207.

7. Oosawa, Y., _J.Phys. Chem.,_ 1984,$\underline{88}$,3069.

8. Herrman, J.-M.; Mozzanega, M.-N.; Pichat, P., _J.Photochem.,_ 1983,$\underline{22}$,333

9. (a) Draper, A.M.; Ilyas, M.; de Mayo, P.; Ramamurthy, V., _J.Am.Chem. Soc.,_ 1984,$\underline{106}$,6222. (b) Al-Ekabi, H.; de Mayo, P., _J.Phys.Chem.,_ 1985,$\underline{89}$,5815. (c) Al-Ebabi, H.; de Mayo, P., _J.Phys. Chem,_ 1986,$\underline{90}$,4075. (d) Al-Ekabi, H.; de Mayo, P., _Tetrahedron,_ 1986,$\underline{42}$,6277. (e) Hasegawa, T.; de Mayo, P., _Langmuir,_ 1986,$\underline{2}$,362.

10. Anbar. M.; Meyerstein. D.; Neta, P., _J.Phys. Chem.,_1966,70, 2660.

D17

Development of ACexpert 2: Implementation of an Expert System for
Automated Metal Analysis by Atomic Absorption Spectrometry

Martin J. Stillman, Timothy Cox and William R. Browett

Department of Chemistry, University of Western Ontario,
London, Ontario, CANADA, N6A 5B7

195

1 Abstract

Expert systems are likely to be of considerable use in assisting the chemist in solving analytical chemistry problems in the future. A major role for expert systems will be in presenting expert knowledge in readily accessible forms, such as, random-access manuals and chemical methods advisors. In order to make rapid progress towards encoding chemical knowledge, the analytical chemist must make use of expert system shells. These software tools provide a programming environment that allow a chemist to design and build complex expert systems quickly and easily, without having to design the low-level support programs. In this paper, we describe our progress in designing an expert system that will control the analysis of metal ions by atomic absorption spectrometry. The system will also be able to offer advice on chemical procedures that could be carried out in order to optimize the analytical values. The first two stages in the development of **ACexpert**, the specification and prototype stages, will be described in detail.

2 Introduction

Increasingly, complex instrumental analysis equipment is viewed as being a "black box". The chemical and procedural limitations of a specific technique may not be well known to the operator. In the analytical service laboratory this means that analyses may be carried out incorrectly. However, it is clear, that as greater productivity is demanded, there will be less time and manpower available to assess the chemistry that is actually being carried out.

Although procedural programming[1,2] is routinely employed to monitor and control analytical instrumentation, this type of program is inadequate for the development of computer programs that will incorporate analytical chemical expertise that is required to monitor and assist each step of an analysis. The most significant restriction is that conventional programming does not efficiently support the ability to manipulate non-numeric, judgemental, or uncertain information that is required to complete an analysis[3]. Typically, analytical procedures combine several types of symbolic reasoning and

problem solving, such as data interpretation, prediction, and diagnosis, as well as instrument monitoring and control[4]. Prior to the introduction of expert system shells, the development of expert system software required the commitment of a programming team that included, a knowledge engineer, a trained programmer, an expensive computer system, and an expert in the technical area. With the availability of the expert system shell on microcomputers, the knowledge engineer, expert, and programmer may be one and the same person.

The minimum configuration for an expert system that can be used as an advisor in the analytical chemistry laboratory, is (i) the user interface, (ii) the inference structure, and (iii) the domain knowledge or expertise.

The determination of metal concentrations using atomic absorption spectrometry with robotic sample preparation and solution introduction, was selected as a test system for implementation of an expert system. We have devised a concept of the automated laboratory, in which computer systems like **ACexpert** will act as an advisor and assistant to the analytical chemist in completing the analyses. Our prototype expert system advisor for metal determination by AAS, **ACexpert**, will comprise the two experts **ACassurance** and **ACanalyst**; in turn, these expert systems are made up of several individual expert systems that carry out specific tasks.

Applications of expert systems within the field of analytical chemistry[5,6,7] fall into two broad categories: (i) systems that are developed to interpret experimental results, and (ii) systems that provide guidance as to the course of action the analyst should take. The challenge in the development of expert systems is to produce a system that will dispense chemical advice at or near the level of a skilled chemist, while at the same time being capable of recognizing that both the skilled chemist and the computer expert system may at times make mistakes[4]. Initially the goal will be to incorporate into the expert system simple, factual, chemical information. However, as chemists become more sophisticated in developing expert systems, the complex rules and procedures, which are needed to describe the dynamic

chemical processes and systems, will be modeled successfully. The
development of an expert system starts with a concept of what an
"expert" is, how the expert performs the scientific tasks, and how the
scientific "expertise" is acquired. There are two main
characteristics that distinguish experts from novices. (i) Experts
are usually expert only with respect to a narrow area of expertise,
and (ii) they rely on shortcuts that they have distilled from their
previous experience[12]. Because distilling the information an expert
uses to make a decision is a time-consumming job, we believe that,
initially, the greatest progress will be made when the expert himself
writes down the rules. Expert systems shells are programs that, in
most cases, provide the user with a structure for the knowledge base,
and provide a means of storing and retrieving the information in the
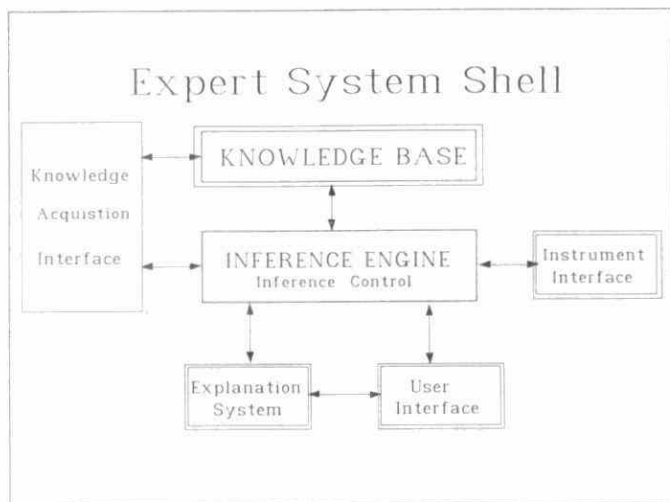knowledge base.

Figure 1.   Structure of an Expert System Shell.

The inference engine is the portion of the expert system that contains the inference and control strategies for the execution of the program. The knowledge base consists of the facts and rules about a domain or the expertise that is incorporated into the expert system. The explanation system provides a detailed explanation of the rules and procedures that were used by the expert system to arrive at the advice. Several types of user interface may also be built into the expert system to input and output data and responses from the user, instruments, or other computers. It should be noted that no single inference strategy, type of knowledge base, etc. will be appropriate for all applications, but rather the inference strategy, interface method or knowledge base structure will have to be designed or selected to match the needs of each specific application.

A simple knowledge base will be installed into the memory of the computer together with the simple rules about the relationships between attributes and values. In a more sophisticated knowledge base, more complex rules about the relationships between objects and structures that are collections of attributes and values, and rules that would be used to examine and control the performance the expert system while it is being consulted[9], will be included. This type of knowledge base would be stored on disk and would be accessed in a much slower fashion.

A good match between the system and the user is vital[8]. It is the task of the user interface to handle all communication between the user and the expert system. The way that information is presented to the user should conform to the user's model and expectations of the task[12]. In selecting or developing a user interface two features are important[8]: (i) The form in which information is elicited and explanations given, needs to be tailored to the intended user -- be they novice or super-expert. (ii) An efficient mode of system-user interaction must be selected that is appropriate to the user's level of skill and understanding.

Special interfaces, such as to instruments or to communication devices, may also be needed to present information to the expert system or respond to the advice of the expert system. In a very sophisticated system, instrumental interfaces may also be used to control an experiment in order to provide information that will be incorporated into the knowledge base, such as the limiting values of a rule.

3 Background to ACexpert

Determination of metal ions using AAS requires the completion of several tasks, each of which requires extensive knowledge and expertise. Although a series of decisions must be made in order to carry out analyses successfully, the procedures and the methodologies of the analytical expertise are well understood. For this problem the three most important criteria for the successful development of an expert system are fulfilled: (i) this is a significant problem for which there is a demand for a solution, (ii) acceptable expert system development tools are available, and (iii) the expertise which will be used to create the expert system is readily available from a variety of sources.
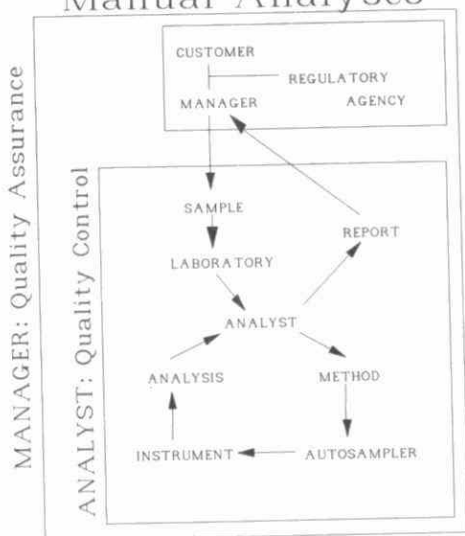
Manual Analyses

Figure 2.   The Flow of Information and a Sample through the
Laboratory: Manual Analysis.

ACexpert models the organization presented in Figure 2.   The
MANAGER and ANALYST make all the decisions and perform all the tasks
that are involved in completing an analysis, as outlined in Figure 3
below.   Typically, the MANAGER consults with both the CUSTOMER and the
REGULATORY AGENCY to determine the method that is to be used.   The
quality control and quality assurance programs and the report writing,
although assisted by computers, are carried out manually as separate
tasks by the MANAGER and the ANALYST.   Once the method has been
defined, the sample preparation is completed by the ANALYST.
Following sample preparation, a portion of the analysis sample, which
contains the analyte, will usually be placed in an autosampler for
automated introduction into the instrument that measures the light

absorbance and determines the concentration of metal by empirical comparison to a calibration curve for that analyte. For the AAS technique, the ANALYST checks the precision of the replicate readings, and using controls, checks the accuracy of the instrument over both the short and long term. Matrix effects, physical interferences, and spectral interferences are among the ANALYST's expert knowledge which is used in combination with checks of the standard methods in the quality assurance program that is used to certify that the result is reliable.

The tasks of the analyst and the manager of the laboratory, as outlined in Figure 2, will be redefined with the incorporation expert systems into the laboratory procedures. The tasks, which are performed by the expert system, typically will be rudimentary and repetitive. Nonetheless, these tasks require the judgement and expertise of an analytical chemist or a skilled technician. The array of expert systems will be incorporated into the normal functions of a fully automated laboratory information management systems. This software is intended to perform the following tasks.

 (i) To reduce data transcription errors by having a single-point of entry for all data, and eliminate the manual entry process of analytical results.

 (ii) To reduce information processing turnaround by automating tedious tasks that are better done by computers, such as reviewing calculations and data transcriptions, which in turn, will make information available faster outside the laboratory.

 (iii) To optimize personnel resources by shifting the most talented staff from easily automated tasks, such as data review, to solving problems that require the expertise of the expert.

ACexpert is described as a set of expert systems shown in Figure 3. Each expert system will perform a separate task and each will be capable of acting independently or as a module of the full system. ACexpert is made up of a hierarchy of expert systems. First,

ACassurance and **ACanalyst,** which in turn are subdivided into individual expert systems that perform specific tasks. As in Figure 2 the flow of information and the sample through the analytical laboratory, begin with the receipt of the sample from the customer by the manager and is followed by the completion of the each of the required tasks. Each expert system is listed in bold typeface and is named beginning with the letters "AC".

In this scheme, the ANALYST's role fundamentally changes from one of a technician to that of a supervisor of the expert system, **ACanalyst.** However, the MANAGER's role remains the same, although he or she now has the assistance of both the ANALYST and **ACexpert** in order to monitor the completion of analyses.

ACassurance is the quality assurance expert system that will be used by the MANAGER and the ANALYST to assist in the execution of a laboratory quality assurance program. Incorporated in this expert system are modules that will be used to provide instruction and advice to the laboratory personnel in carrying out their tasks and completing the analysis procedures thoroughly. **ACanalyst,** the quality control and process control expert system, will comprise five separate expert systems that will be used in methods selection, process control, analysis, fault diagnosis, and quality control. The functions of the individual expert systems are outlined below.
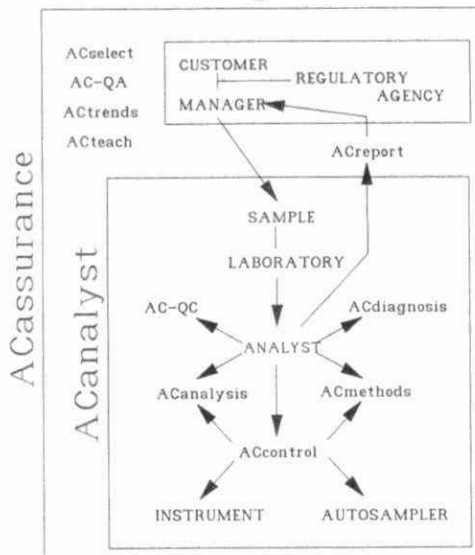
ACexpert

ACsurance

ACanalyst

ACselect
AC-QA
ACtrends
ACteach

CUSTOMER
REGULATORY AGENCY
MANAGER

ACreport

SAMPLE

LABORATORY

AC-QC   ACdiagnosis

ANALYST

ACanalysis   ACmethods

ACcontrol

INSTRUMENT   AUTOSAMPLER

Figure 3: **ACexpert** is a chemical analysis expert system.

In the revised model of the flow of information and the sample in the laboratory, **ACexpert** provides an environment within which the MANAGER, ANALYST, and analytical instruments interact to complete the analysis. In the way that we have specified the **ACexpert** system, the CUSTOMER and MANAGER will begin using the **ACassurance** expert system to assist them in selecting the appropriate methods and analysis criteria that are consistent with the requirements of the REGULATORY AGENCY and the capabilities of the laboratory. The analysis criteria and the recommended methods would then be available to **ACanalyst**, for using the selection of specific chemical and instrumental procedures and "standard methods" that are to be used by **ACcontrol** to complete the

metal determination of each sample. In normal operation, the ANALYST would manually confirm the method selected by **ACmethods** and monitor the performance of the analyses. All other aspects of the analysis would be carried out automatically, although still under the direct supervision of the ANALYST.

We have concentrated on defining and protyping two parts of the **ACexpert** system: **ACmethods** and **ACcontrol**. The specifications for **ACmethods**, as a random access analytical procedure manual, are presented below. Similar specifications would need to be developed for each of the expert systems that make up **ACexpert**.

Table 1: **ACmethods** specifications and requirements.

1.	rule based expert system
2.	explanations for finding procedures
3.	ability to use ambiguous information
4.	multiple answers
5.	conditions to be satisfied, (analyte, regulatory body, analytical technique, sample type, etc.)
6.	inference technique
7.	hardware specifications
8.	integration with other expert systems

We have used two shells in our work, namely, KDS3 and CxPERT. At a minimum, a shell must have a knowledge representation scheme, an inference or search mechanism, a means of describing the problem, and a way to determine the status of a problem while it is being solved[15]. Of the many types of expert systems[14], several are required to complete the structure of **ACexpert**: consultation (**ACmethods**), diagnosis (**ACdiagnosis**), monitoring and control (**ACcontrol**), interpretation (**ACanalysis**, **ACtrends**), planning (**ACselect**), and instruction (**ACteach**).

Consultative or diagnosis experts, may be used for methods selection or fault finding (**ACmethods, ACdiagnosis**), and typically are built with rule-based shells that require consistent relationships between causes and symptoms, or conditions and conclusions, as well as the ability to identify these relationships. The decision making is made more difficult, if symptoms are masked by other symptoms, the symptoms are intermittent, the data is inaccessible, or if there is uncertainty and lack of knowledge about the relationships between the causes and symptoms[4].

Interpretation type of expert systems (**ACanalysis, ACtrends**) define a situation by analyzing acquired sensor data or data in a database in order to determine its meaning[4]. This type of expert system requires that a known and consistent interpretation be obtained from a given set of data. In most cases the system must be rigorously complete in that a solution is only provided, if the system is absolutely sure of the interpretation. The primary difficulties are incomplete data, long reasoning chains, unreliable data, and reasoning with contradictory data.

In the monitoring and control type of expert system (**ACcontrol, ACreport, AC-QC, AC-QA**), observations are compared to expected or desired results[4]. The performance of a physical system is analyzed, then the expert system takes the appropriate action in order to produce a desired level of performance. For real-time monitoring and control of critical instrumental functions fast information input and decision making ability are the primary requirements. An inherent advantage of a monitoring and control expert system is that it does not know whether it is talking to a real process or a simulated one. By attaching the expert system to a high fidelity simulation model, a wide variety of anomalies can be introduced[16]. The expert system can be readily improved or adapted for instructional purposes.

4 ACmethods

The **ACmethods** prototype is based on the Environment Canada NAQUADAT dictionary of Parameter Codes (1986) for the selection of the metal determination method for an Aluminum analyte in aqueous samples.

The prototype advice is presented to the user as simple text. For this demonstration prototype strict adherence to the content of the NAQUADAT manuals was observed. However, as the expert system evolves, the analytical chemist will be required to tailor the **ACmethods** manual to the needs of the local laboratory, as well as the other expert systems. Furthermore, as the system evolves and is integrated into **ACexpert,** the textual presentation of recommendation and detailed advice would be inappropriate for the completed system that will require an automated system of information transferal and validation.

The first prototype of **ACmethods** uses a small set of attributes or conditions (analyte, regulatory body, analytical technique, sample type, sample preparation method, or detection limit) to resolve the NAQUADAT methods. As the **ACmethods** expert system grows in complexity this set of attributes will need to be expanded.

5 Implementation of ACmethods with an Inductive Expert System Shell

The first prototype of **ACmethods** was implemented with the KDS3 development system which is an inductive expert system shell. This shell, like many other inductive shells, generates rules from examples. The developer enters a large number of examples for the machine's information base into the development system. The development system then uses an algorithm to convert the examples into a rule or number of rules and to determine the order that the system will follow when questioning the user and making a recommendation[9]. Unlike other production, rule-based systems, KDS does not deal with attributes or values, but only conditions, which are input into KDS as text strings that are either true, false, or known with some degree of certainty[13]. To KDS, every entry is simply a string of text that has some factors associated with it and a certain place in the logical hierarchy, as seen in Table 3.

The KDS environment for expert system development, provides the developer with substantially the same interface and performance characteristics as the end product that the user will encounter. Therefore, testing is an on-going process. The system developer or user answers YES, NO, or ? (don't know or don't care) to conditions,

which have already assigned in the program, according a specific case history that is being considered. If no conclusion can be found with the specified answers to the conditions or an ambiguity arises because of an answer during the system development process, then the developer is requested to supply the answer.

Table 2: The KDS-based **ACmethods** questions and responses that are required for KDS **ACmethods** to select Environment Canada NAQUADAT method 13050.

#	KDS-based **ACmethods** Questions	Answer
1.	Analysis of dissolved metal in an aqueous sample?	No
2.	Analysis of mineral acid extractable metals?	No
3.	Use the flame AAS technique?	Yes
4.	Analysis of metal in suspended material of sample?	No
5.	Analysis of metal in sediments?	Yes
6.	Metal extracted with solvent to eliminate interferences?	No
7.	Required by the Water Quality Branch of Environment Canada in Ottawa?	Yes

An example of the use of an analytical methods selection expert which was developed with the KDS3 system is shown in Table 2. To use **ACmethods**, the user selects a set of attributes and values or conditions which best describe the characteristics of the sample and analytical method. In this example, the user is attempting to select a method for the determination of total concentration of aluminum in a sediment sample, according to the requirements of the Water Quality Branch of Environment Canada in Ottawa. From the main menu, aluminum, Al, is selected as the analyte of interest. The use of a menu to present conditions, is useful when mutually exclusive options are to

be selected. The KDS shell then prompts the user with a series of
questions that can be answered with Y(yes), N(no), or ?(don't know or
don't care).

The standard screen interface, which is presented to the user,
appears as in Figure 4. A series of these screens, which contain
conditions that the developer considered to be important in order to
select an analytical method, are presented to the user for validation
of the conditions. In the right hand panel, a further description of
the condition, or added information is presented to the user. The
final screen of the KDS-based **ACmethods** prototype is presented in
Figure 4.

Title: Actinium to Carbon	Sample type: total sediments
	Sample prep.: Open digestion with HNO3, and HF.
THIS IS MY BEST ANSWER. 13053 Al. Flame AAS, total sed., required by A-WQB-O.	Analysis: Flame atomic absorption by direct aspiration. measured spectrophotometrically at 309.3 nm and compared with identically prepared standards. An acetylene-nitrous oxide reducing flame is used. Detection limit: 1 mg/kg Required by: WQB Ottawa. **** Approved WQB Method ****

Figure 4: An analytical method selected from **ACmethods**
implemented KDS. The title refers the title of the expert
system module. The answer, in this case method 13053, is
restricted in length to the two lines that are seen above.
An expanded description that accompanies the answer is
presented in the right hand panel.

If question 7 in Table 2 was answered ambiguously with a ? (don't
know or don't care), then two answers or methods would be presented
and the system would warn the user that insufficient information was
provided to resolve the differences, as seen in Figure 5. Since only
one method, either method 13050 or 13053, can be used at any one time,
this ambiguity would have to be resolved before the other expert
systems which are part of **ACexpert** or the human technician could
complete the Aluminum determination.

```
┌─────────────────────────────────┬───────────────────────────────┐
│                                 │                               │
│  Title:  Actinium to Carbon     │                               │
│                                 │ You may press the BACKSPACE key│
│                                 │ if you wish to go back and see │
│  Possible conclusion(s):        │ unresolved conditions which you│
│  1  13050 Al, Flame AAS, total  │ previously answered with a "?".│
│     sed., required by A-WQB.    │                               │
│  2  13053 Al, Flame AAS, total  │ Conditions to which you already│
│     sed., required by A-WQB-O.  │ gave a Y or N answer will not be│
│  I have been unable to resolve  │ asked again except the last one.│
│  1 condition(s) either directly │                               │
│  from the answers or through    │                               │
│  inference. As a result         │                               │
│  please regard the above as     │                               │
│  conjecture, not advice.        │                               │
│                                 │                               │
└─────────────────────────────────┴───────────────────────────────┘
```

Figure 5: An ambiguous response from **ACmethods,** which was implemented using KDS, will result in two methods 13050 and 13053 being selected. In this instance the shell provides routines that acknowledge that two or more conclusions could be reached with the given set of conditions. In the right hand panel of the screen the expert system provides instructions on how to resolve the ambiguity.

In this example, the expert system presents the user with both menus and individual questions during the course of the session in order to select the appropriate method. In a more complex expert system the KDS standard interface could have been replaced with a customized user interface or an instrument interface, if real-time data input and analysis were desired.

6 Implementation of ACmethods with a Rule-based Expert System Shell

When the performance of the expert system is important, such as in real-time instrument control or in a customized user interface, a very low-level procedural programming language, such as C, may have to be used. However, a significant drawback to the use of the language C is that considerable programming is required to build the very high-level constructs that are needed for expert system development[13].

For these situations, expert system shells provide mechanisms for directly linking C routines into the system. Alternatively, the developer can use a specialized shell that has been designed specifically to allow the development of the expert system using C language. Typically, these shells generate C code as output. This C code along with code, which has been written by the developer, can be combined and compiled into a working expert system.

Two examples of prototypes of **ACmethods,** which were generated with the CxPERT shell, are presented below. The most significant differences between them is that the first uses the traditional depth-first, backward-chaining logical structure to search the knowledge base for a solution, whereas the second uses a breadth-first, forward-chaining search methodology to search for a solution. Assuming that the programmer is familiar with programming in C, it is a rather straightforward process to learn the CxPERT language, add the appropriate customized C routines, and then compile and link the routines to form a fast working expert system.

The backward-chaining, depth-first **ACmethods,** which was developed with CxPERT, like the KDS-based **ACmethods** expert system, has been implemented using the Environment Canada NAQUADAT dictionary of Parameter Codes (1986) for the selection of the metal determination method for an aluminum analyte in aqueous samples. However, CxPERT requires the developer to directly construct the rules of the expert system. Procedure 13053, from the NAQUADAT dictionary is shown in Figure 6. The CxPERT key words are highlighted in bold. The procedure presents the rule that must be tested to validate the selection of the hypothesis in two different ways. The first, which is delimited by the keywords **rule/endrule**, presents the English interpretation of the rule, and the second, which is delimited by the keywords **if/then/endif**, presents the CxPERT rule that must be validated.

Each of the clauses in the **if/then/endif** rule will be tested in succession until either a contradiction is found or all of the clauses are validated. Each rule in the knowledge base of the analytical procedures was constructed to have attributes in the order of:

analyte, sample type, technique type, and then regulatory agency, sample preparation technique, or detection limit, if the values of these attributes will help to uniquely resolve each procedure. The attributes, which will be used to test each rule, are designated with the suffix "_test". Once a search has been initiated, the compiled expert system knows which the attributes are unassigned, and will successively query the user until all the attributes of the rule are assigned. If, during the course of this query of attributes, a false response is received, then the expert system proceeds to subsequent procedures, and tests the attributes of the subsequent rules with the assigned values, until all the unassigned attributes are satisfied or no procedure in the knowledge base is found that satisfy the assigned attributes.

In the example below, Table 3, the backward-chaining, depth-first, CxPERT-based **ACmethods** will query the user for the analyte of interest, Al, in this case. Answering the question according to Table 3 will result in the advice that the NAQUADAT procedure 13053 should be used. Most questions are answered with reference to a menu of selections.

ACmethods, then proceeds by testing the first procedure of the system; the procedure for NAQUADAT method 13001. The first condition of the rule of procedure 13001 (Is the element Aluminum?) is true. However, this procedure fails on the second condition (Is the sample type "Total"?), because the sample type of this example is "sediments". The expert system searches its knowledge base until a procedure is found for which the sample type "sediments" is selected. In this case control of the program then passes to the procedure for method 13050, as the first procedure for which the sample type "sediments" is true. The third condition of technique type is answered as "Flame AAS" which is also true for procedure 13050. The fourth condition, which is the selection of a detection limit, in procedure 13050, fails when the answer 1000 micro-grams per Litre is compare to 500 in the knowledge base. Control is then passed to procedure 13053, which shown in Figure 6, for which this answer gives a true response. The subsequent questions, which are generated by the

rule of procedure 13053, are answered and validated. As a result, this CxPERT expert system suggests that NAQUADAT procedure 13053 would fulfil the conditions specified.

Once a procedure has been resolved, the advice is displayed, and the user is asked if the search of the knowledge base should be continued to look for other procedures that may also conform to the conditions that have been specified. A comprehensive list of procedures that satisfy the specified attributes can only be achieved by instructing the expert system to continue its search through the knowledge base, in effect requesting a breadth-first search.

Table 3:   The CxPERT-based **ACmethods** questions and responses that are required for CxPERT **ACmethods** to select Environment Canada NAQUADAT method 13050.

	CxPERT-based **ACmethods** Questions	Answer	Procedure
1.	What element do you wish to analyse for ?	Al	13001
2.	Sample type ?	sediments	13001
3.	Technique type ?	Flame AAS	13050
4.	What is the detection limit (in micro-grams) ?	1000	13053
5.	Regulatory agency ?	Environment Canada, WQB	13053
6.	Water Quality Branch Section ?	Ottawa	13053

```
begin
   rule
   ^If you want determine the concentration of Aluminum, Al in a
   sediment sample and you will use a Flame AAS technique and
   according to WQB Ottawa and with a detection limit of 1000
   ug/kg.
   Then use the NAQUADAT method 13053 as outlined below.
   endrule
   IF equal(element_test, "Al")
      and equal(sample_type_test, "Sediments")
      and equal(analysis_technique_test, "Flame AAS")
      and equal(regulatory_agency_test, "WQB Ottawa")
      and detection_limit_test eq 1000
   then
      advise
         NAQUADAT Method:   13053
         Element or Compound:  Al, Aluminum
         Sample Type:  total sediments
         Sample Preparation:  Open digestion with HNO3, HClO4, and
HF.
   Analysis:  Flame atomic absorption by direct aspiration,
measured spectrophotometrically at 309.3 nm and  compared with
identically prepared standards. An acetylene-nitrous oxide
reducing flame is used.

         Detection Limit:  1 mg/kg in sediment
         Results In:  mg/kg
         Required By:  WQB Ottawa
                    *** Approved WQB Method ***
      endadvise
      put("yes" into found)
      reset(more)
   endif
endprocedure.
```

Figure 6:   The backward-chaining, depth-first, **ACmethods**
CxPERT rule which defines the procedure for the NAQUADAT
method 13053. The information delimited by **rule and endrule**
can, at the user's request, be presented on the screen. The
actual CxPERT rule, which will be tested, is delimited by **IF**
and then and can also be displayed by the user for an
explanation of the state of the expert system. Finally, the
advice, which will be dispensed when each condition of the
rule is validated, is delimited by **advise and endadvise.**

7 ACcontrol: The monitoring and control expert

ACcontrol is a system that must function in real-time using procedures for instrumental control that are described in the next section. This expert system must be structured so that it has access to the appropriate information at the right time from the sensors of the instrument, and structured so that decisions are made quickly. Specifically, the decision-making and supporting calculations must occur within the cycle time for the sampling. Combining the monitoring, control, and interpretation tasks of the expert systems requires that individual tasks be performed at the same time, either on separate computers, which can communicate with each other, or within a multi-tasking environment on a single computer. In either case, a great deal of importance is put on computational and reasoning efficiency. Generally, the system must have complete information to allow the interpretation expert to return a definite answer to the controlling expert. The occurrence of incomplete information, long reasoning chains, unreliable data, and contradictory input information are difficult for this type of fault finding expert and send an alarm to the operator.

8 Instrument Control

Before the monitoring and control expert can be implemented, a traditional procedure base user interface, a data base manager, and instrument controlling programs must be produced. The hardware of the ACexpert system consists of a Gilson 222 autosampler and a 401 dilutor, and a Varian 875 AAS that are all controlled by an AT-compatible microcomputer. The autosampler and dilutor act in concert to provide solution preparation and sample injection into the AA spectrometer. The user interface makes extensive use of graphical displays to control the instrument setup, sample setup, and metal determination procedures in an interactive environment based on MS Windows (Microsoft Corporation). This graphical user interface is similar to the type depicted as a "Human Processor Interface for ICP-AES" by Karanassios and Horlick[17] or as presented for the automation of a chromatography workstation[18].

9 User and Instrument Interface Development

The user and instrument interface programs have been created as a set of applications under MS Windows that can be run at the same time within the MS Windows multitasking environment. Each MS Windows application has access to all the features of MS Windows such as pull-down menus, and mouse driven windows and icons. Figure 7 shows the subsidiary tasks that **ACcontrol** operates. Because menus are used, any number of these functions may be active at any time. All of these features are necessary to provide a simple to use, robust system for instrumental control. It is worth mentioning here that Windows is in fact not a true multitasking system. In other words, there is no scheduling program that ensures a particular application won't use all of the CPU time. Rather, it is up to the application to yield control to the other applications periodically.
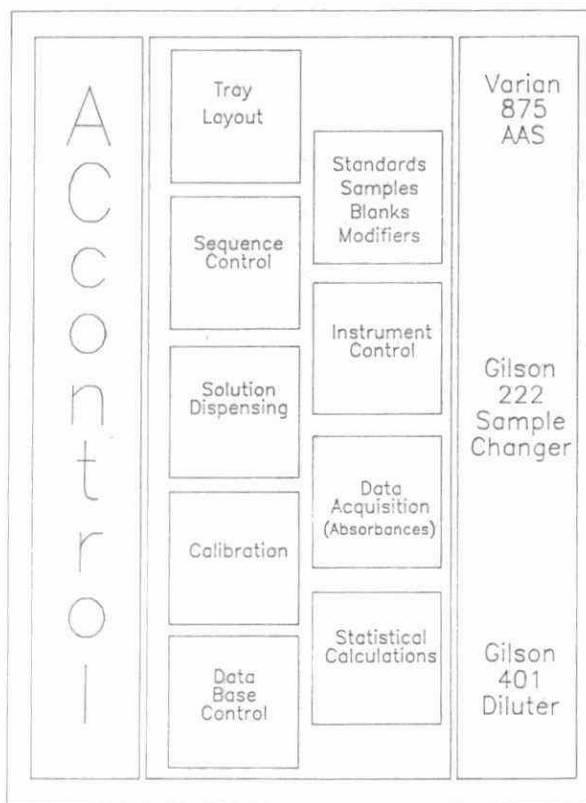
Figure 7. Tasks under control of **ACcontrol**.

Instrument control, sample preparation, and sample injection into the AAS are performed by two MS Windows applications. The first, called AAS, is responsible for providing a computer interface to the Varian 875 AAS. This application provides the user with a "Control Panel" (see Figure 8) that will allow complete control of the AAS operating parameters. Using a mouse, the user simply clicks the appropriate control button on the screen and the application (through a RS-232 hookup) sends the AAS the command. The user is able to save the instrument setup in a disk file which may be retrieved for future use or printed on a printer.
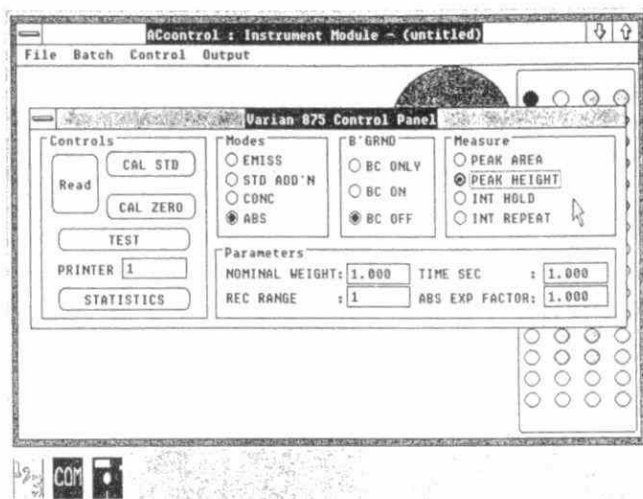
Figure 8. **ACcontrol:** simulation of the control panel of the Varian AA spectrometer.

The second application, called Sampler, is the heart of the instrumental control application. This application sets up three window procedures to handle (i) control of the Model 222 Autosampler and the Model 401 dilutor as slave devices, (ii) the sequencing of the samples to be analysed within a batch, and (iii) the data values received from the AAS following every measurement. Control of the Gilson devices is carried out as follows. Each device is assigned a unit number on the GSIOC, (similar to the IEEE specifications) and the computer acts as the master device. Each device is programmed in it's primitive mode. This mode allows all of the features of the device to be used. Sample placement is performed by providing the user with a picture of the rack they will use from template designs stored for a number of racks and beakers, the user identifies the appropriate rack, which is then drawn on the screen. By using the mouse the user designates which tubes contains the standards, samples, blanks, or controls. Also, containers for larger amounts of blank or reslope solution may be put on the sampler tray, these too are displayed on the computer screen in the way they appear on the sampler tray (see Figure 9). The data-feedback-window procedure reads the data that appears on the RS-232 line from the AAS and dispatches the data to two other procedures: (i) data output and (ii) the quality control procedures. The data output procedure writes the data to the screen, the disk file or the printer. The quality control procedure will later calculate the sample's relative standard deviation and decide if the sample should be reanalyzed.
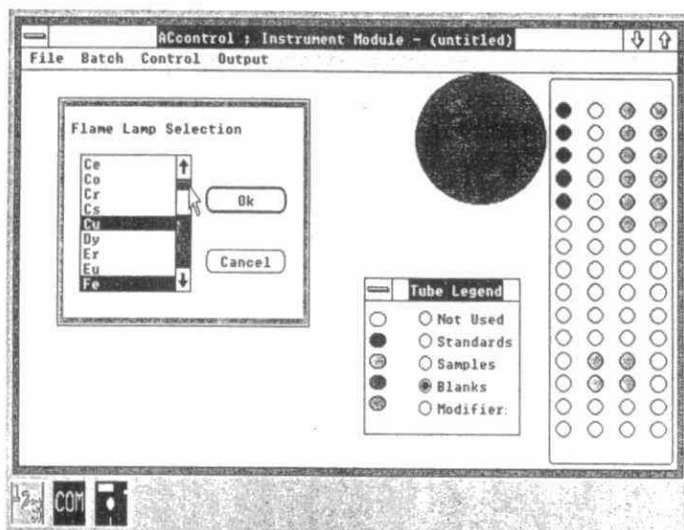
Figure 9. ACcontrol: layout of the autosampler.

Once tubes have been allocated, the database is completed with textual entries for each sample, batch ID numbers can be added, and incremental numbers can be assigned automatically, Figure 10. The elements to be analysed for in each tube are assigned from a default list of available lamps. For standards, the concentration is also given at this step, Figure 11. Advice, "Help", is available from a "cook book" of standard procedures, arranged according to the lamps in use. The Gilson pump can be set up with both flow rate and aliquot size, allowing full control over the aspiration of the sample into the flame, Figure 12.

Figure 10. **ACcontrol:** entry of textual information.
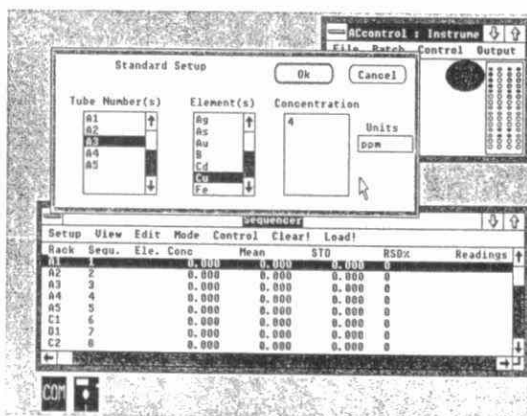
Figure 11. **ACcontrol:** assignment of elements to specific tubes.
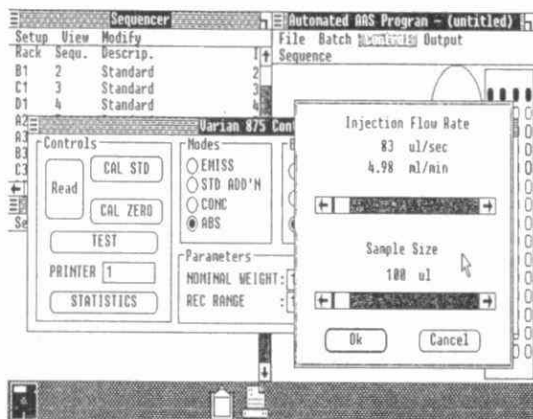
Figure 12. **ACcontrol:** settings for sample injection.

The data base holds all the set up information, and arranges the operating sequence so that all tubes with the same element are analysed in one batch. The user, or **ACcontrol** can adjust the sequence. For each analysis, the signal graphics, the phototube response as the sample enters the flame, is recorded, Figure 13. This trace can be recorded from any point in time after the start of the sample selection by the autosampler, so that effects due to the pump operation on the flame stability can be observed. The graphical information can be stored and used in assessing the quality of the AAS itself, by injecting, air, water, and a standard, and comparing these results with data obtained under assured conditions. Finally, the calibration curve can be compared with stored averages of previous calibartion curves for this element in this matrix, Figure 14.
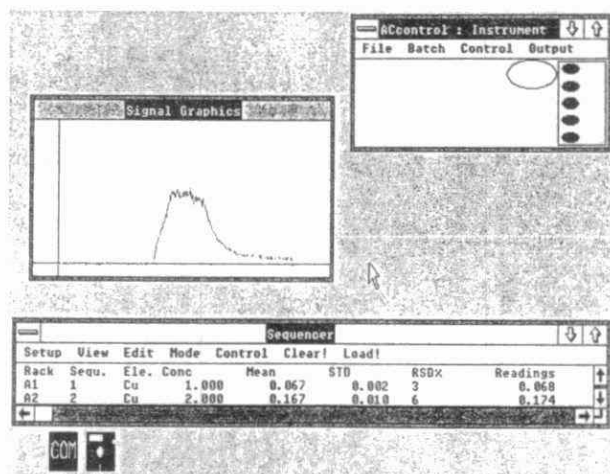
Figure 13. **ACcontrol:** signal graphics captured in real time.
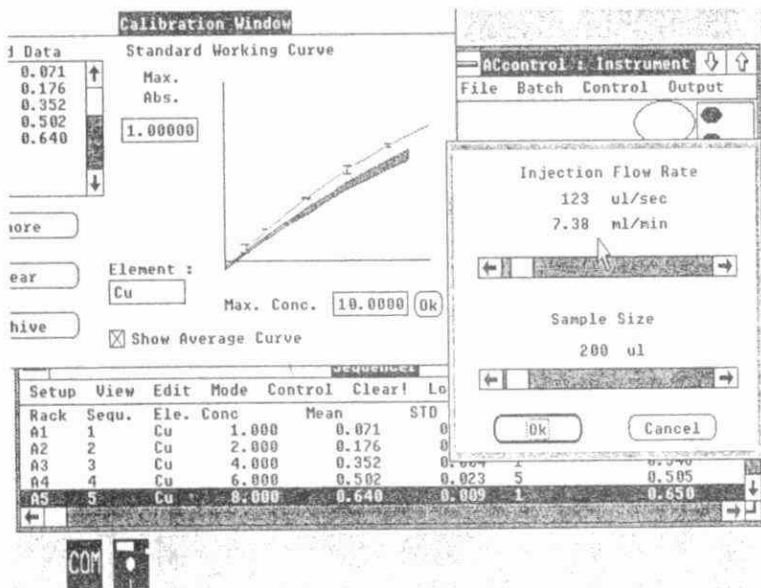
Figure 14. **ACcontrol**: comparison between a working curve and archived average curves.

10 Discussion

This paper describes the design and implementation of **ACexpert**. Clearly, the control aspects of this project required a large effort. The goal of **ACcontrol** was to provide a completely accessible control module such that information obtained from the other modules could be used to interrput the assigned schedule allowing for repetitive sample analysis and unscheduled quality control programs to be started. In this manner we expect that the next development will involve the addition of the real-time assessment of the data being measured with respect to the quality of the signal graphics.

The significant research involved is the encoding of the expert knowledge such that it becomes accessible to the user in an easy to use form. **ACmethods** will be the key module in the role of advisor. Both KDS3 and Cxpert provide a useful environment for coding this information. The important feature of our development to date is that we believe that the validation of the information and the design of the user interface, may in the end, out weigh the advantages and disadvantages of any particular shell. For example, Cxpert requires provedural programming innthe form of access to a "C" compiler, so it is a much less attractive shell to start working with when compared to the KDS3 environment. However, after some use, it turns out that it is organising the knowledge that becomes the most difficult taks, not the writing of the linking code.

12 References

1. T. Nyokong, and M.J. Stillman, <u>J. Auto. Chem.</u>, **8**, 122 (1986).

2. Z. Gasyna, W.R. Browett, T. Nyokong, R. Kitchenham, and M.J. Stillman, <u>Chemometrics</u> (1988) in press.

3. R. Bach, J. Karnicky, and S. Abbott, "<u>Artificial Intelligence Applications in Chemistry</u>", T.H. Pierce, and B.A. Hohne, eds. American Chemical Society, Washington, 1986, Pg. 278-296.

4. B.P. Butz, <u>Abacus</u> **5**, 30, 1987.

5. T.P. Bridge, M.H. Williams, and A.F. Fell, <u>Chem. Brit.</u>, **23**, 1085 (1987).

6. F.A. Settle, Jr., M. Pleva, C. Booker, H. Iams, D. McClintock, and T. Moore, <u>Amer. Lab.</u>, **March**, 17 (1987).

7. V. Berry, <u>Amer. Lab.</u>, **April**, 126 (1987).

8. P.E. Slatter, "<u>Building Expert Systems: Cognitive Emulation</u>", Ellis Horwood Ltd., West Sussex, England, 1987.

9. P. Harmon, R. Maus, and W. Morrissey, "<u>Expert Systems Tools and Applications</u>", John Wiley & Sons Inc., Toronto,1988.

10. H. Schildt, "<u>Artificial Intelligence Using C</u>", Osborne McGraw-Hill, Berkeley, CA, 1987.

11. R. Benson, "<u>If/then AI using Lotus 1-2-3</u>", If/then Solutions, San Francisco, CA, 1987.

12. K. Parsaye, and M. Chignell, "<u>Expert Systems for Experts</u>", John Wiley and Sons Ltd., New York, 1988.

13. E.R. Tello, "<u>Mastering AI Tools and Techniques</u>", Howard W. Sams & Cmpy. Indianapolis, USA, 1988.

14. C. Townsend, "<u>Mastering Expert Systems with Turbo PROLOG</u>", Howard W. Sams & Co., Indianapolis, IN, 1987.

15. R. Citrenbaum, J.R. Geissman, and R. Schultz, <u>AI Expert</u> **2**, 30 (1987).

16. D. Leinweber, K. Gidwani; 1986 IEEE Westerb Conf. on
    Knowledge-based Engineering and Expert Systems, G.S. Robinson,
    ed., IEEE Computer Society, Washington, 1986; pp 69-73.

17. V. Karanasslos, and G. Horlick, Applied Spectr. **41**, 360, 1987.

18. Henshall, J.A. Schibler, Amer. Lab. **September,** 42, 1987.

D18

ADAPTATION OF WATER PRECONCENTRATION TECHNIQUES FOR TRACE METAL DETECTION. B.R. Hollebone, E. Dowdall*, K.L. Singfield, L.J. Brownlee, Department of Chemistry, Carleton University, Ottawa, Ontario, K1S 5B6; P. Vijan, Laboratory Services Branch, MOE.

Introduction

The overall goal of this project is the design, construction and verification of a water sampler for the preconcentration of trace metals from raw and treated drinking water, in preparation for analysis by inductively coupled plasma emission spectroscopy (ICPES). This has been approached through the adaptation of an existing preconcentration system for organic pollutants in raw and treated water developed in these laboratories, and currently under test by the Ministry of the Environment. A schematic of the water sampler is shown in figure 1.

To use ICPES effectively for water analysis and to take advantage of its simultaneous multi-element capability, simultaneous preconcentration of as many as possible target trace metals is required. The target metals are a group of potentially toxic metals found in raw and treated waters such as aluminum, chromium, cobalt, copper, iron, lead, manganese, mercury, molybdenum, nickel, strontium, vanadium, and zinc. The water samples also contain other metals at much higher concentrations such as sodium, potassium, calcium, and magnesium. In terms of toxic risk, these can be called irrelevant. Their presence, however, can cause serious matrix interferences in ICPES analysis.

229

Successful trace analysis of heavy metal ions requires the elimination of irrelevant but interfering hard metal ions. In this proposed preconcentration procedure, the separation of the target metals from the irrelevant metals is being achieved through selective adsorption on a modified resin contained in a column. The resin is modified by the addition of a chelating ligand. Essentially, the column can simultaneously provide an adsorbing medium for the target trace metals and an eluting medium for the irrelevant metal ions. Traditional methods involve collection of all metals, followed by the selective elution, coprecipitation or extraction of the metals of interest.

The differentiation of the irrelevant hard and the trace soft metal ions is possible because of their different complexing characteristics. The hard alkali and alkali-earth metals exhibit a preference for a hard donor atom, such as oxygen, for complexation. The soft heavy metals exhibit a preference for a soft donor atom, such as sulfur or nitrogen. Therefore, preferential complexation can be employed to separate hard from soft metals. To ensure that the undesirable metals are continuously eluted during the concentration of the heavy metals, the ligand should have soft nitrogen and sulfur donor atoms preferably used in combination with each other in a polydentate ligand.

The ligand-to-resin bond can be formed by varying the resin type and manipulating the terminal functional group on the ligand, to achieve various binding mechanisms. An adsorbent link

230

is most applicable for the methodology desired in these studies, which requires fast batchwise resin preparation, flexibility in binding and elution of metals, and choice of elution products. In the desired binding mechanism the bidentate or chelate ligand is hydrophobically adsorbed to the resin to form metal complexes by covalent donor bonds. The adsorption of the ligand to the resin is strengthened by an electrostatic attraction between the hydrophobic aromatic structure of the resin and the hydrophobic aromatic portion of the ligand.

Some commercially available ligands that are possible candidates for the desired extraction are 2-mercaptopyridine, 2-quinolinethiol, 2-mercaptothiazoline and 2-mercaptobenzothiazole. Most recent literature has shown the ability of 8-mercaptoquinoline to preconcentrate micro amounts of elements in natural water. These elements include a wide range of metals from both group #2 and #3[1].

The type of resin chosen to be tested will be XAD-4. This resin has been successfully used to support hydrophobic chelate ligands which coordinate using either 2 sulphur atoms or 1 sulphur and 1 nitrogen atom[2].

Experimental

The overall design of the trace metals preconcentration instrument is the same as that of the organic sampler. The major

emphasis in adapting the design to one suitable for metal analysis is on the replacement of stainless steel components with Teflon or other organic materials such as nylon, polypropylene or polyethylene.

Initial tests were performed to determine which ligand yielded the optimum ratio of ligand to resin. Other considerations for the choice of ligand were the ease of resin preparation and shelf life of the loaded resin. The ligand chosen was 2-mercaptobenzothiazole (MTB) since a ratio of 0.5 mmol ligand:1 ml resin provided the maximum concentration of ligand complexed on the resin. This ratio presents 2.5 mmol of ligand for every 5 ml of resin. There is sufficient amount of ligand on the XAD to completely complex with the metals since a 40 ppb metal sample in a 25 ml solution represents approximately $1 \times 10e-8$ moles of metal.

Experimentation was performed using glass columns packed with 5 ml of the ligand loaded resin. All materials were soaked in concentrated nitric acid and thoroughly rinsed with deionized/distilled water. A 50 ml cocktail of five heavy metals — cadmium, chromium, zinc, copper and lead (1ug each) — and one of four undesirable metals — potassium, sodium, magnesium or calcium (10 to 300 ppm each) — in deionized/distilled water was applied to the columns. The columns were then washed with 50 ml of deionized/distilled water to simulate actual sampling conditions and to determine the retention of the hard and/or soft metals on the column. The columns were then eluted with 25 ml of

1% nitric acid and the samples were stored in polyethylene
bottles. Solutions of methionine or cysteine in 5% nitric acid
were also tested as possible eluting agents.

Results

The initial experiments of the column containing 100 grams
of treated resin were performed with a 1 mL test sample
containing 1000 ppb of cadmium. After a 50 mL wash and a 1 %
nitric acid elution, essentially 90 % recovery was obtained.

Similar conditions were used to test the effects of
interferences on five heavy metal ions. The range of
interference concentrations used was from 0 to 300 ppm, while the
concentration of test metal ions - chromium, copper, zinc,
cadmium and lead - was 40 ppb. The results for interferences
from magnesium and calcium as functions of their concentrations
are shown in figures 2 and 3. Recoveries in the presence of
sodium and potassium interferences were not affected
significantly compared to interferences by calcium and magnesium.

The recoveries of test ions following elution are variable
with and without the presence of interfering ions. Cadmium and
lead exhibit approximately 100% recovery in almost all cases
while copper, chromium and zinc exhibit much lower recoveries,
with the highest recoveries occurring at the lowest concentration
of the interfering ions. Copper exhibited the lowest recovery

even in the absence of interfering ions.

The extracts were analyzed for the presence of these interfering ions and none were found, indicating that these ions were not bound to the ligand at the time of elution.

Discussion

The adaptation of the dioxin preconcentration sampler to the detection of trace levels of heavy metal ions has involved several major considerations. The first is the conversion of all internal surfaces from stainless steel to inert plastics to minimize the adsorption of metal ions or hydrated metal oxides to any hydrophilic surfaces. Another, is the adaptation of the filtering step to insoluble metal detection. Finally, of much importance, is the development of a suitable ligand for soluble metal ion detection.

The first step is in progress and the fully adapted preconcentration instrument will be functional in the near future. The second step requires only the identification of filter materials similar to those used in the organic sampler which will not interfere with mildly acidic extraction processes required for metals. The filtration step is optional and if desired, the water stream can be taken directly to the adsorption columns to avoid this step. The adaptation of the column adsorbent procedures is the most important feature of this research. Essentially, a technique is being developed to be

6

transparent to irrelevant ions and thus, selectively capturing the heavy metal ions of interest. The adsorbent is treated with ion selective ligands in an effort to preferentially adsorb the trace ions at the 40 ppb level in a background of 10 to 300 ppm of alkali and alkaline earth metal ions.

The results show that MTB is a very effective chelating agent for some of the test metal ions. It does not appear to bind either the monovalent alkali metal ions or the divalent alkaline earth ions but does appear to bind the target metal ions. This can be deduced from the observed recovery behaviour.

In the absence of interferences, copper, zinc and chromium divalent ions yield low recoveries while those of cadmium and lead tend to be highest, particularly in the absence of interfering ions. This correlates with the very strong bonding of cadmium and lead to sulphur, and the preference of chromium, copper and zinc for bonding to ligands containing oxygen or nitrogen. These latter ions are more easily complexed by either water or the oxygen-containing nitric acid ion than are cadmium and lead. This situation is unchanged in the presence of sodium or potassium because these ions have very weak bonding to sulphur ligands.

Thus, according to the data, it does appear that MBT is effective as a selective chelating agent for the cadmium and lead ions which are most strongly bound by sulphur, from a water matrix, even in the presence of interfering ions. The ions of chromium, copper and zinc are not completely retained for two

reasons. First, in the absence of interfering ions the ligand is not efficient at retaining these metals during the water wash used to remove the interfering ions prior to acid elution. Second, in the presence of interfering ions there is a further "salting out" effect which increases with increasing interference concentrations.

The 1% nitric acid medium is adequate for effective elution of the test ions. In revising the experiments, more retentive ligands must be studied to permit significant capture of the harder copper, chromium and zinc metals. This would require ligands with more readily available thiol groups, replacing the mercapto ligands or possibly amino groups rather than aromatic nitrogen atoms. In any case, the work on this first ligand has provided evidence that the concept of selective trace metal ion capture is viable, and further work to optimize the ligand structure should permit the effective analysis of most toxic metals at or below the ppb level.

References:

1/   Yu. A Bankovsky, M.V. Vircavs, D.E. Vereris, A.R. Peline, and D.K. Vircava, Talanta, 1987, 34, 179-182.

2/   J. Chwastowska and E. Mozer, Talanta, 1985, 32, 574-576.

8

FIGURE 1

METAL RETENTION
EFFECT OF CALCIUM

Metal Recovered (ppb) 100% = 40ppb

Ca (ppm)

— Cr   — Cu   — Zn   — Cd   — Pb

FIGURE 2

METAL RETENTION
EFFECT OF MAGNESUIM

Metal Recovered 100% = 40 ppb

Mg (ppm)

— Cr   — Cu   — Zn   — Cd   — Pb

FIGURE 3

SCHEMATIC OF MARK II APS
- - - - ELECTRICAL CONNECTION
PG   PRESSURE GAUGE
ARV   AIR RELEASE VALVE
PS   PRESSURE SWITCH
FS   FLOW SENSOR
CV   CHECK VALVE
NCV   NORMALLY CLOSED VALVE

D19
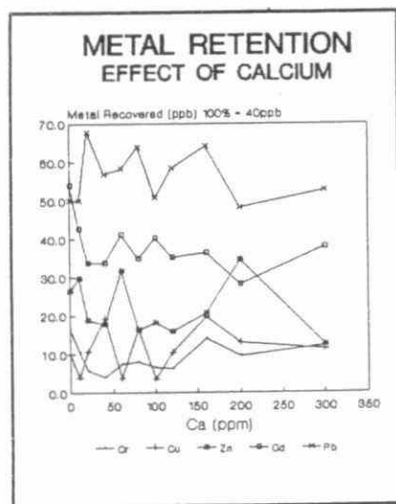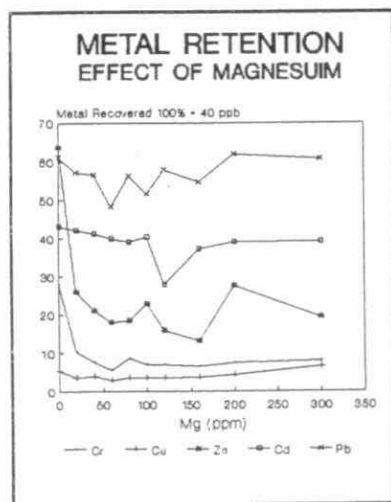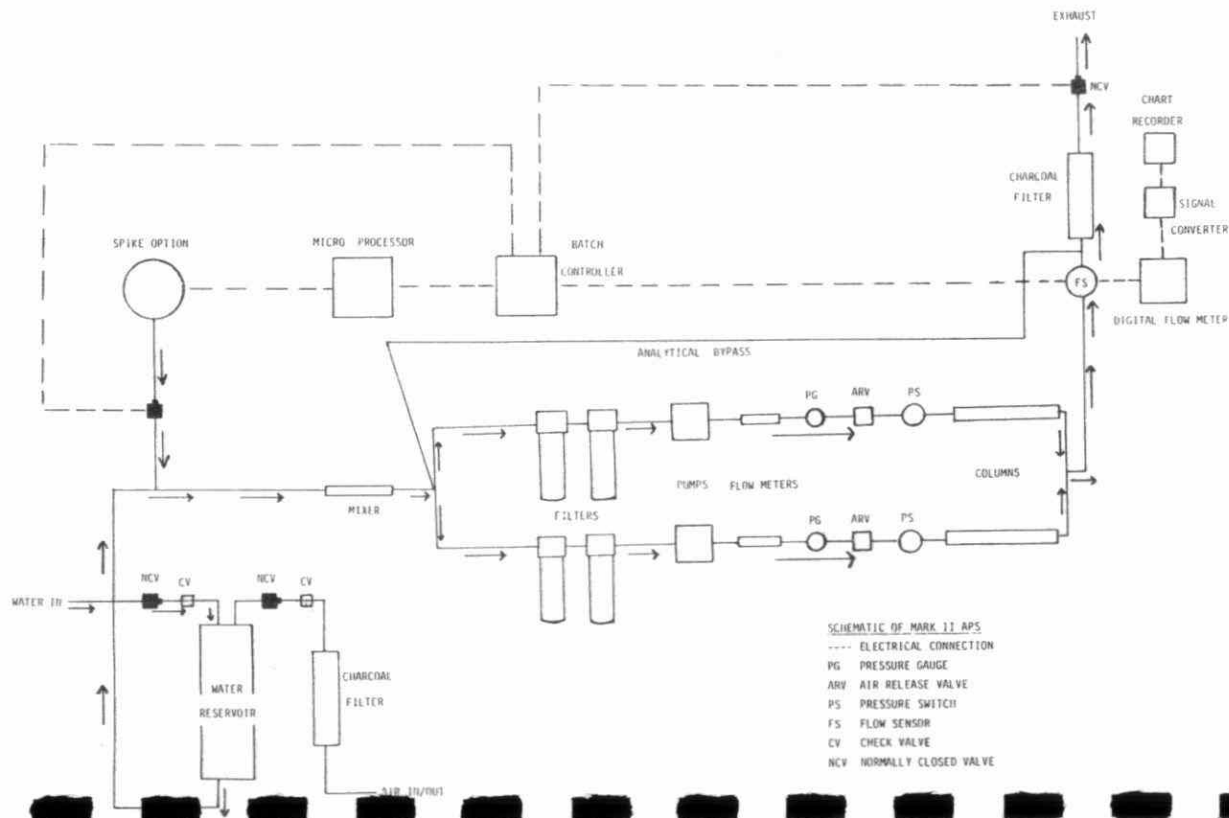
Comparison of Various Leachate Extraction Procedures
for the Characterization of Inorganics in Wastes

by James R. Kramer, Jill Gleed, Pierre Brassard and Pamela V. Collins, Department of Geology, McMaster University, Hamilton, ON L8S 4M1

Introduction

The characterization of the mobility of potentially toxic metals in wastes is important because metal mobility is generally related to toxicity. Therefore considerable effort has been put forth to determine a procedure which will accurately predict metal leachability under the wide range of conditions expected in a waste depository. In addition, many industrial wastes could be used for a number of engineering purposes (e.g. ballast, fill etc) if the waste could be shown to be stable.

The model for leachate mobility that has been widely adopted has been taken from studies by the U.S. Environmental Protection Agency (1). This scenario assumes that industrial wastes will be mixed in a limited amount with municipal mostly organic matter. It is further assumed that the organic portion will be oxidized to organic acids, and that acetic acid is a suitable model substance. Thus the resultant acetic acid will leach to some degree metals contained in the rest of the waste. Assuming the industrial waste is a certain fraction of the total waste (e.g. 15%) and further assuming that the industrial waste has the base buffering capacity equivalent to $Ca(OH)_2$, one can calculate the equivalents of (acetic) acid equal to the base equivalents. The procedure, and various modifications thereof (2), requires the mixing of a measured portion of waste dispersed in water over a period of about 24 hours with periodic adjustment of the pH to 5.0. After the waste has reacted, an aliquot is filtered, and key metals are measured. If the concentration of these metals exceeds various criteria, the waste is labelled as being hazardous. In essence, Ontario has adopted this procedure and has written code 309 which specifies the leachate extraction procedure in detail. In addition, criteria for metals and other substances are listed.

Any extraction procedure, by definition, is **operationally defined**. Some important variables to consider in the above procedure include:

*   effect of varying the solid concentration
*   variability of pH related to amount and rate of acid addition

* effect of heterogeneous phases. Acid attack may expose a more active solid by removing coatings etc., or a strong sorbing phase may be exposed which would decrease the metal concentration in solution.
* time for equilibrium or steady state to be reached.

This report describes the effect of some of the above factors on the metal extraction of a number of wastes. Furthermore, it compares the metal mobility determined by the LEP with that obtained under a constant pH (typically 5) and with metal mobility from the first two steps of a sequential extraction procedure (3).

Finally a more rigorous and arduous analysis of metal mobility was carried out. This procedure resolves the concentration of acidic functional groups from which the acid buffer index function (β) can be derived (4). Secondly, the solid is titrated with metal at constant pH to determine the binding capacity of a specific metal. Copper was used as the model metal.

In the evaluation of the various procedures two criteria are suggested:

1) When different techniques give different metal mobilities, the technique releasing the most metal would result in a cautious evaluation. BUT "to err on the side of safety", one must also ask if the procedure is representative of a possible environment at the waste site.

2) How different are the results for different tests? If there is a wide variation in results, one may question the value of any method. If quite different techniques give similar results, then one may have more confidence in the procedure.

Methods

Leachate Extraction Procedure (LEP): The LEP, as prescribed by the Ontario Ministry of the Environment, is well-documented (2). In brief, a solid sample of 50 g/L concentration is rotated end-over-end at 10 rpm for up to 24 hours. At prescribed periods of time, the pH of the sample is measured, and the pH is adjusted with a prescribed amount of acetic acid. At the end of the mixing time, a sample is filtered, and metals are analyzed flame atomic absorption spectroscopy, using the method of standard additions (AAS). Metal concentration criteria for an acceptable leachate are given in code 309.

Sequential Extraction Procedure: The Tessier *et al.* (3) sequential extraction procedures was carried out on representative waste samples. In this method, a one

gram sample is treated with more aggressive reagents, and after each treatment a suite of metals is analyzed. The first two treatments are similar to the LEP technique. Extraction 1 is an "ion exchange" step involving the release of metals by $MgCl_2$; Extraction 2 is a "carbonate" reaction involving reaction of acetic acid buffered to a pH of 5.

A modification of the method was carried out to simulate slightly reducing conditions. This environment was achieved by carrying out the sequential extraction after the system had been completely purged with nitrogen to remove oxygen from solution and solid surfaces.

Constant pH extraction: A sample of variable concentration is brought to a constant pH (typically 5) using an Instrument Development Group (Burlington, ON) (IDG) automated double channel titrimeter. The sample is maintained at the prescribed pH within 1 0.02 units. Filtered sub-samples are taken periodically and are analyzed by AAS for a suite of metals. In addition, the change in copper concentration under pH stat conditions was monitored continuously using a Cu-electrode.

Both the acid neutralizing capacity (ANC) of the solid mixture and the metal leachate concentrations are determined.

Discrete Affinity Spectrum Analysis: This technique involves the analysis of titration data carried out for about 40 hours using an IDG automated titrator. The analysis recovers the pK(acid)-concentration spectra or pK(metal)-concentration spectra. In brief, the theory for data reduction is as follows: Assume that the multiple functional groups of a solid can be represented as **additive mono-metal multi-ligand** sites. These assumptions can be symbolized as: $M + :S_i = M:S_i$, where M is the metal (e.g. H^+, Cu^{2+} etc) and $:S_i$ is the ith reaction site. Furthermore for a mono-metal assumption, $pK_i = - \log K_i$, $K_i = [M][:S]/[M:S]$, $C_i = [M:S_i] + [:S_i]$; and by substitution, $[:S_i] = C_i K_i/([M] + K_i)$, and $[M:S_i] = C_i [M]/([M] + K_i)$. Brackets-[ ], represent molal concentrations. The ion balance equation can be used for an acid/base titration to give:

$$[ANC] + [BASE] - [ACID] = \Sigma [:S_i] = \Sigma C_i \frac{K_i}{[H] + K_i} \tag{1}$$

where [ANC], [BASE] and [ACID] are the respective acid neutralizing capacity concentrations, base and acid titrant concentrations. For a metal titration, the mass balance expression can be used:

$$[\text{TITRANT}] - [\text{M}] = \Sigma C_i \ \frac{[\text{M}]}{[\text{M}] + K_i} \tag{2}$$

Equation 1 and 2 are solved by linear programming (LP) techniques to preserve the condition, $C_i \geq 0$. Furthermore, the error condition, L_1 (5), the sum of the absolute of the error, is used, because it is shown to be more robust for large amounts of data, and the results is less biased by a few poor data points (6).

Results: LEP and Tessier Extraction Comparison

Table 1 summarizes the results obtained for the LEP and Tessier sequential extractions for a variety of industrial wastes and for a variety of metals. There are some patterns that come out of this analysis of different methods. First there is little or no difference between the Tessier (steps 1 & 2) and the Tessier (steps 1 & 2) with nitrogen purging. Therefore it does not appear worthwhile to continue work on the nitrogen purging method, especially since it is labour intensive. There is often a significant difference between the LEP and the Tessier extraction, as shown by the bolding in table 1. In every case, the Tessier method gives the higher concentration. As discussed below, this is probably due to the lack of attainment of a constant pH in the LEP. There are two points worth noting in this conclusions: (1) the data are normalized to the mass of the solid, since the Tessier method requires this, and (2) there is no means for predicting when the Tessier method will produce a greater metal concentration. Undoubtedly the complex heterogeneous phases present in the wastes is the reason for this variability in results.

Results: Effect of pH for Extraction

One sample, X-pert sludge, was characterized for metal mobility as a function of time and for pHs of 4.5, 5 and 6. In this study, the IDG titrimeter pH stat mode was used to control the pH at the selected pH. When the system indicated that the pH was stable (usually 0.1-0.5 hours), the experiment was started. Aliquots of samples were taken at various times. Figure 1 shows the pH dependent leachate results for six metals over time. In general, as the pH decreases, the metal mobility increases, as would be expected. Chromium is the one exception where the Cr mobility is maximum at a pH of 5. In many cases, the differences between the metal extraction at pH of 5 and pH of

Table 1. Trace metals in industrial wastes. Comparison of results for the LEP method and the Tessier method with and without nitrogen purging. All results are in μg/g of dry solid equivalent. Tessier steps 1 & 2: (1): Tessier steps 1 & 2 with nitrogen purge (2); LEP: (3). Significantly higher results (10 times the lowest) are noted in bold.

Steel and Foundry Slags:

	BOF Slag#2			BOF #7			DOF Slag		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
Ni	19	**25**	2	**20**	**18**	3	10	**21**	2
Cd	1	**4**	0.3	**2**	**2**	0.2	2	2	0.5
Pb	35	42	9	30	**33**	3	7	5	1
Cu	7	**10**	1	5	6	1	7	5	1
Zn	6	7	1	4	5	1	25	10	17

	Slag			Electric Furnace			S. BOF slag		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
Ni	38	46	27	11	**18**	1	17	18	4
Cd	0.7	0.3	0.1	0.9	0.2	0.3	3	3	0.4
Pb	19	30	12	23	**31**	2	38	35	6
Cu	19	14	53	5	4	0.6	6	6	0.2
Zn	18	20	6	5	**121**	1	4	6	3

Foundry dusts, brass foundry, mold dusts etc:

	Brass Mould Sand			Brass-exhaust dust			Brass-ZnO dust		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
Ni	**145**	17	9	**511**	349	6	21	22	7
Cd	**2**	1	0.1	18	15	5	1100	1100	1060
Pb	**4020**	3700	140	**15,500**	14,400	69	14,400	13,200	10,000
Cu	**24,800**	55	26	**122000**	54900	92	3500	3000	1210
Zn	4250	2470	1220	**23800**	16100	670	247000	226000	>200000

Sludges and Chemical Wastes:

	Wallaceburg sludge			Rieke Sludge			X-pert Sludge		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
Ni	474	499	248	29	32	18	23	18	6
Cd	**4**	3	0.1	17200	17000	4100	6	7	5
Pb	23	19	6	124	141	18	26	35	6
Cu	375	374	406	36	47	8	**441**	417	12
Zn	865	**921**	80	25000	25700	11800	14200	145000	33000

	Cobalt Refinery		
	(1)	(2)	(3)
Ni	23	29	11
Cd	2	**3**	0.3
Pb	36	16	5
Cu	**4630**	4530	327
Zn	**227**	215	4

4.5 is small. This suggests that the pH of 5 is a good choice for leachate extraction. It is disturbing to note, however, that often the system does not reach steady state or equilibrium in about 25 hours. In figure 1, cadmium, copper and nickel do not seem to have reached a stable value in the alloted time.

Results: Effect of Particle Concentration

The effect of solid concentration upon metal mobility is shown for two different wastes and two metals in figure 2. For both samples and both metals, increasing particle concentration decreases metal mobility. Also the effect appears to be quite significant. It is noted that iron and manganese tend to give the opposite results, as the particle concentration increases, the soluble metal concentration increases. It appears that particle concentration is an extremely sensitive parameter in the assessment of metal mobility.

Results: Affinity Spectrum Analysis of Acid Buffering

It is a common observation that all solid waste samples act as very strong acid/base buffers. Therefore in the LEP or any other procedure where there is not continuous pH control, there will be large changes in pH due to the surface of the particles reacting with the acid. In order to assess the acid/base surface chemistry of the wastes, an acid titration was carried out over various time periods, and the resulting data sets of 50-200 points was analyzed using discrete affinity spectrum analysis as discussed earlier. The titrations extended from initial (pH 8 to 11 depending upon sample) to a pH of 3. The data were analyzed for a 0.5 pK interval and are summarized in table 2.

The data are interpreted qualitatively as follows. The sample will tend to buffer the solution to a pH equivalent to the pK when the concentration for that pK is large. This conclusion is readily gleaned from the buffer intensity (4), which is:

$$\beta = 2.30259 \left\{ [H] + [OH] + \Sigma\, C_i \quad \frac{[H]\, K_i}{([H] + K_i)^2} \right.$$

The analysis included short times of titration (30 minutes) and long times (30 hours). It is quite apparent that time of reaction is a very important parameter, both because there is a great deal more ANC developed with increasing time and also there are

Figure 2.   *Effect of particle concentration upon the mobility of cadmium and copper for X-pert sludge and a brass foundry ZnO dust. All samples were extracted at pH of 5 and at 10 rpm rotation.*

X-pert sludge:
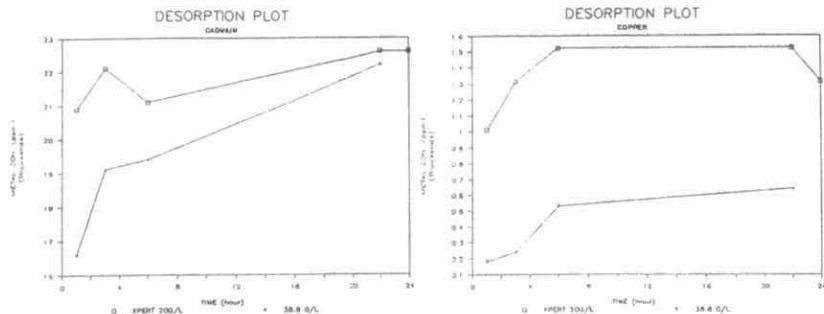
Brass foundry dust:
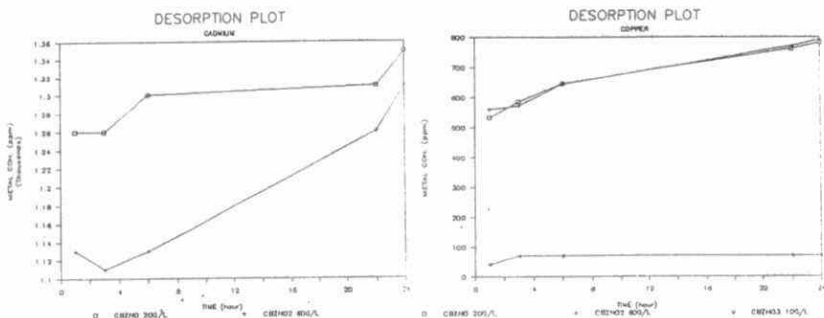
Figure 1. *Effect of pH of extraction upon metal mobility. All analyses are for solution concentration and for a particle concentration of 25 g/L.*
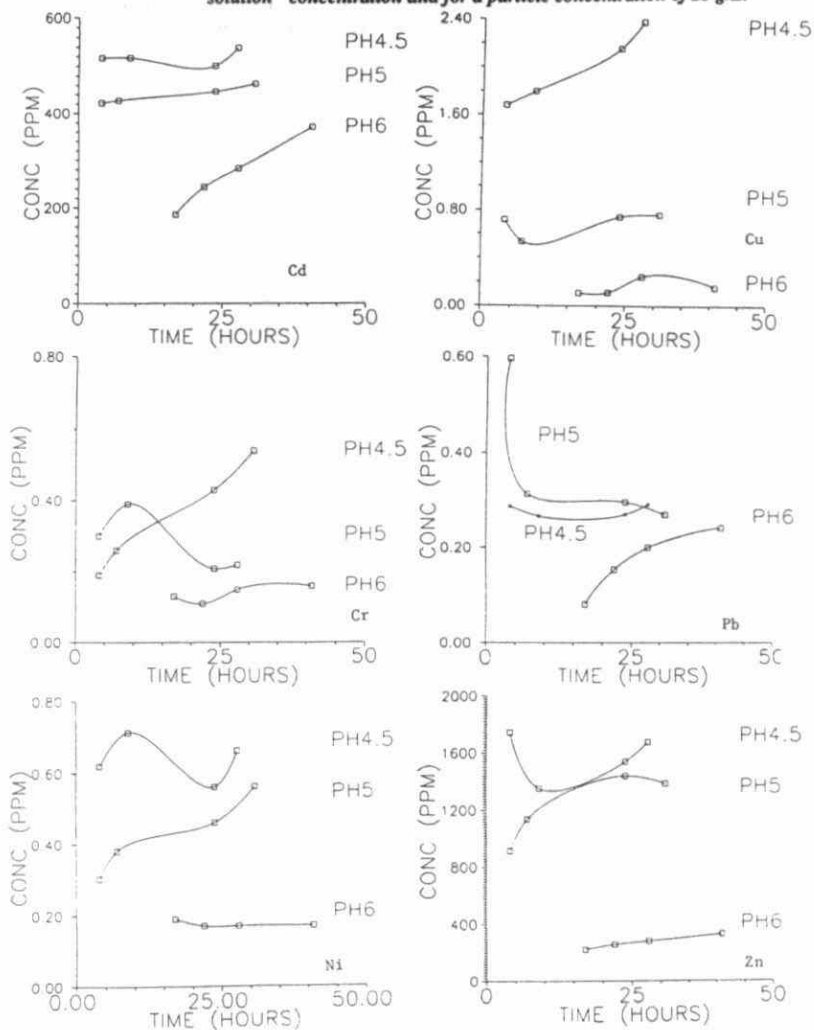
Table 2.   *Affinity Spectrum Analysis of Industrial Wastes. All titrations were carried out in 0.04 N ionic strength medium. Concentrations are given in μmol/L.*

pK	BOF Slag: 1g/L: 30 min Concentration	BOF Slag: 1g/L: 30 hr. Concentration	X-pert Sludge 1g/L: 30 min Concentration	X-pert Sludge 0.7g/L: 30 Hr. Concentration	Wallaceburg 1g/L: 30 hr Concentration
3	1132	2463	0	3640	0
3.5	0	6305	0	0	248
4	106	0	259	47	1716
4.5	1086	0	2107	1689	0
5	0	3324	0	0	0
5.5	0	0	0	49	0
6	0	0	387	3058	0
6.5	0	0	154	0	0
7	53	0	58	0	3030
7.5	802	4170	0	0	0
8	0	0	72	0	0
8.5	0	0	0	0	0
9	0	0	0	0	0
9.5	0	157			
10	321	2083			
10.5	1609	0			
11	0	1216			
ANC:	4317	19,720	3010	7474	8252

different pKs developed over time. For example, the BOF slag shows a difference of over four times in acid neutralizing capacity for the long time compared to the short time titration. In addition to the pKs apparent in the long term titration at low pKs (3, 3.5), there is an additional pK at 5 which shows a zero concentration for the short titration. These results clearly demonstrate the slow equilibration of surfaces with respect to acids and the importance of carrying out titrations very slowly over 30-40 hours. To do this special automated equipment is necessary, but any other result will not be representative of the reaction of the waste with its environment.

Results: Continuous Analysis of Copper at Constant pH

The dual channel IDG system was used to hold the pH constant at 5 while the copper was monitored continuously with a copper electrode in the second channel. Figure 3 shows for a sample of Wallaceburg sludge the response of copper (electrode mv which is proportional to log of the copper concentration) as an acidic titration is carried out over a period of about 24 hours and to a pH of approximately three. The copper concentration continues to rise (electrode potential decreases) as would be

Figure 3. Plot showing the change in pH over time with the slow rate of addition of acid and the change in copper concentration as monitored by ion specific electrode. The electrode response is shown in mVs which are proportional to the logarithm of the copper concentration.
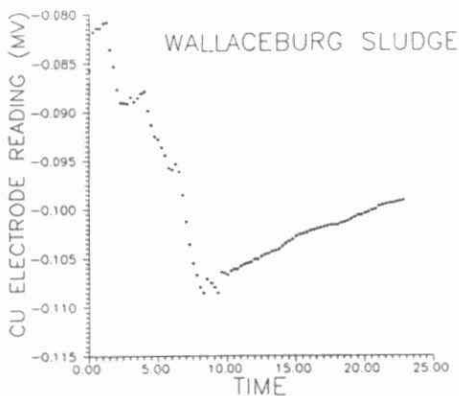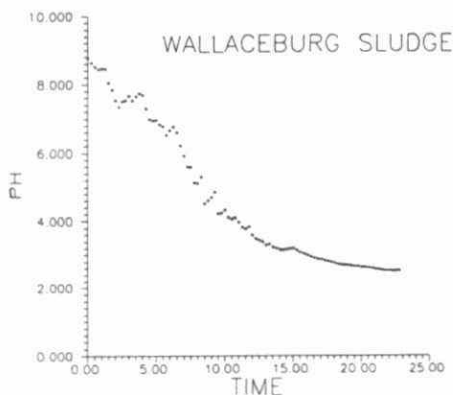
expected, but at a pH of about 4, the copper concentration reverses, indicating a new phase reaction occurring. This shows the kind of reversal that may occur when dealing with a heterogeneous phase system.

Summary:
1) Comparison of the LEP procedure shows that it often underestimates the metal mobilized in comparison to the Tessier extraction procedure (steps 1 and 2). This underestimation is undoubtedly due to the lack of a constant pH in the LEP.
2) There is little or no difference in the results from the Tessier (steps 1 and 2) with or without nitrogen purging. It is recommended that the nitrogen purging procedure not be used due to the more intensive effort required.
3) There is a pronounced effect due to variation of the solid concentration. Normally the more concentrated samples release lesser metal to solution.
4) Detailed short and long term titrations indicate that a minimum of 20 hours and preferably 50 hours is required to obtain a true steady-state indication of equilibrium.
5) Affinity spectrum analysis of acid and metal titration data gives a more quantitative and more rigorous indication of metal mobility. The technique should be experimented with further.

References
1. U.S. Environmental Protection Agency, Office of Water and Waste Management, "Test Methods for Evaluating Solid Wastes", SW-846 (1980).
2. "Leachate Extraction Procedure", *in* Environmental Protection Act, Regulation 309 (1980).
3. Tessier, Andre; Campbell, P.C.G.; Bisson, Marc. "Sequential extraction procedure for the speciation of particulate trace metals", *Anal. Chem.*, 51:844-851(1979).
4. Stumm, Werner; Morgan, J.J. *Aquatic Chemistry*, Wiley-Interscience (1981), p160.
5. Chvatal, V. *Linear Programming*, V. Klee(ed), W.H. Freeman and Co., NY (1983).
6. Claerbout, J.F.; Muir, F. *Geophysics*, 38:826-844(1973).

D20

Immunoassays for the Detection of 2,4-D and Picloram in River Water and Urine

J. Christopher Hall*, Raymond J. A. Deschamps, and Kim K. Krieg

Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Abstract. Immunoassays for 2,4-D [2,4-dichlorophenoxyacetic acid] and picloram [4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid] detection were developed using polyclonal antibodies raised in New Zealand White rabbits. Concentrations of 2,4-D from 100 to 10,000 ng/mL and from 50 to 10,000 ng/mL could be quantitated with an indirect enzyme-linked immunosorbent assay (ELISA) and a radioimmunoassay (RIA) in river water and urine, respectively. Concentrations of picloram from 50 to 5000 ng/mL also could be quantitated in river water and urine by RIA. Determinations using the immunoassays required no sample clean-up. Specificities of the antisera for structurally similar herbicides such as MCPA, 2,4,5-T, dicamba, clopyralid, and triclopyr were low compared to 2,4-D or picloram. The RIA methods incorporated a novel radiolabel consisting of [3H]glycine covalently linked to the herbicide molecule. The RIA was considered more reliable than the ELISA. The immunoassays would be suitable for herbicide quantitation in applicator exposure and environmental fate studies.

INTRODUCTION

The potential of immunochemical technology for pesticide analysis has been examined by Hammock and Mumma (1980) and more recently by Van Emon et al. (1985) and by Cheung et al. (1988). Immunoassays are proposed for pesticides that are difficult to analyze by standard techniques. Many pesticides, including 2,4-D and picloram, require an extensive sample preparation including derivatization before they can be analyzed by gas chromatography. As alternative methods, immunoassays can be sensitive, specific, and precise providing for rapid, cost effective analyses.

Current concerns about potential health hazards connected with pesticide use have focused on 2,4-D as a suspected cancer causing agent (Hoar et al., 1986). As a broadleaf weed killer, 2,4-D is used extensively in field crops, on turf, and in non-crop lands. Its widespread use and associated health concerns have made monitoring environmental and biological samples for the presence of 2,4-D desirable. Among the types of samples monitored are well waters for 2,4-D contamination (Frank et al., 1987) and urine samples for applicator exposure studies (Grover et al., 1986; Libich et al., 1984).

Picloram is used for the control of woody and broadleaf herbaceous plants. It is relatively resistant to breakdown in the environment and has been found to be mobile in the soil (Hamaker et al., 1963). Picloram residues have been found in surface and groundwater samples (Frank et al., 1987; Baur et al., 1972). The mobility in the environment shown by picloram along with the susceptibility of certain crops to extremely small amounts of this compound (Ragab, 1975) make monitoring water for picloram residues necessary.

Radioimmunoassays (RIA) for 2,4-D (Rinder and Fleeker, 1981, Knopp et al., 1985) have been reported. Recently, Fleeker (1987) described two direct enzyme-linked immunosorbent assays (ELISA) developed for the detection of 2,4-D in water. To date, no immunoassays have been reported for picloram. The following report describes the development of an indirect ELISA procedure for 2,4-D and simple RIA procedures for 2,4-D and picloram detection in river water and urine samples without prior clean-up procedures.

MATERIALS AND METHODS

Chemicals and Materials. The analytical standard of picloram and the [2,6-^{14}C]picloram (sp. act. 264 MBq/mmol) were provided by the Dow Chemical Company, Midland, MI. The analytical standard of 2,4-D along with N-hydroxysuccinimide (NHS), N,N'-dicyclohexylcarbodiimide (DCC), 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (CMC), isobutyl chloroformate, triethylamine, bovine serum albumin (BSA), rabbit serum albumin (RSA), goat antirabbit phosphatase, Sigma 104 phosphatase substrate tablets, Tween 20 (polyoxyethylene sorbitan monolaurate), Freund's complete adjuvant, and Freund's incomplete adjuvant were obtained from Sigma Chemical Company, St. Louis, MO. The ^{14}C-labelled 2,4-D {(2,4-dichlorophenoxy)-[2-^{14}C]acetic acid; sp. act. 11.6 GBq/mmol} was obtained from Amersham/Searle, Don Mills, ON. Aquasol 2 and [2-^{3}H]glycine (sp. act. 1609.5 GBq/mmol) were obtained from New England Nuclear Research Products, Boston, MA. Diethanolamine was obtained from Fisher Scientific Ltd., Don Mills, ON.

Instruments. The optical density of microtiter plate well content was read on a Bio-Rad Model 2550 EIA Reader. Liquid scintillation spectroscopy was performed on a Packard Tri-Carb 460C liquid scintillation system.

Buffers. Phosphate buffered saline (PBS) contained 8.00 g of NaCl, 0.20 g of KH$_2$PO$_4$, 2.90 g of Na$_3$PO$_4$ 12H$_2$O, and 0.50 g of KCl per L of distilled water. The pH was adjusted to 7.4 with 1 M HCl. PBS-Tween washing solution was prepared by adding 0.5 mL of Tween 20 per L of PBS. Diethanolamine buffer contained 100 mL diethanolamine per L of distilled water. The pH was adjusted to 9.8 with 1 M HCl.

Water and Urine Samples. River water was collected from the Speed River, Guelph, Ontario. The water was filtered through a Whatman No. 1 filter paper, and stored at 4°C until time of analysis. Human urine was collected from a male donor over a 24 h period, pooled, and stored at 4°C. Water and urine samples were fortified with an ethanolic solution of analytical standard of 2,4-D or picloram to achieve final sample concentrations over the range of 50 to 10,000 ng/mL.

Preparation of Immunogens. Picloram and 2,4-D were conjugated to BSA as described by Fleeker (1987). Equimolar amounts of [^{14}C]picloram (46 mg, 45.5 Bq), NHS (22 mg), and DCC (39 mg) were dissolved in the sequence given in 2.5 mL of dioxane. The solution was allowed to stand at room temperature for approximately 18 h at which time it was filtered to remove the precipitate. The filtrate was evaporated to dryness on a rotary evaporator under vacuum at 35°C. A solution of BSA (500 mg) dissolved in 3 mL of 0.10 M borate buffer (pH 9) was added to the residue and the mixture was agitated gently for 1 h at room temperature. The resulting solution was dialyzed against several changes of deionized water over 36 h at 4 C and lyophilized. The procedure was repeated using [^{14}C]2,4-D (42 mg, 45.5 Bq) in place of picloram. The amount of herbicide bound to BSA was estimated by measuring ^{14}C present in weighed portions of product dissolved in PBS. Approximately 20 and 15 molecules of picloram and 2,4-D, respectively, were bound per BSA molecule.

Antisera. New Zealand White rabbits were injected subcutaneously with an emulsion consisting of 0.5 to 1.0 mg immunogen dissolved in 0.5 mL of PBS and an equal volume of Freund's complete adjuvant. The injections were repeated 3, 6, and 10 days after the initial injection, substituting Freund's incomplete adjuvant for complete adjuvant. A booster injection was given one month after the initial injection and was repeated at monthly intervals thereafter. The rabbits were bled for antibody titer determinations 10 days after each boost. Antisera for 2,4-D or picloram immunoassay development were prepared from a single bleed in each case.

Preparation of Coating Antigen. To a solution of 50 mg 2,4-D (0.23 mmol) in 2 mL of dioxane was added 50 mg CMC (0.12 mmol). The solution was stirred for 2 h at room temperature. RSA (50 mg) was dissolved in 6 mL of 0.1 M borate buffer (pH 9). The 2,4-D solution was added dropwise to the RSA solution over a period of 15 min. The mixture was stirred for 18 h at 4°C and dialyzed against several changes of deionized water.

Preparation of Radiolabels. The mixed anhydride of 2,4-D was prepared by adding 2,4-D (6 mg), triethylamine (5 μL), and isobutyl chloroformate (5 μL) in the sequence given to 500 μL of dioxane. A portion of the mixed anhydride solution (100 μL) was added to a solution of 100 μL of [^{3}H]glycine (0.1 mCi), 100 μL of dioxane, 100 μL of distilled water, and 2 μL of 2 M NaOH. After 1 h, an additional 2 μL of NaOH was added. The reaction was allowed to proceed for a total of 4 h at room temperature.

The 2,4-D-[^{3}H]glycine conjugate was isolated and purified by TLC. The reaction mixture, [^{3}H]glycine, and 2,4-D were spotted on a silica gel plate (Whatman K5F). The plate was developed in a diethyl ether: petroleum ether: formic acid (70:30:2 v/v/v) solvent system to a 10 cm solvent front. The 2,4-D standard and the unreacted mixed anhydride of 2,4-D were visualized under UV light (R_F 0.82). Fractions of the plate were scraped, eluted with 90% EtOH, and assayed for radioactivity. Three fractions contained appreciable amounts of radioactivity: R_F 0.00 (corresponding to [^{3}H]glycine), R_F 0.47, and R_F 0.76. The fractions were assayed for binding in an RIA using antisera known to have anti-2,4-D activity. Only the fraction corresponding to R_F 0.47 showed binding. The binding also was shown to be competitively inhibited with free 2,4-D. The fraction chromatographing to R_F 0.47 was therefore assumed to be the 2,4-D-[^{3}H]glycine conjugate.

The mixed anhydride reaction was repeated using picloram in place of 2,4-D. The picloram-[^{3}H]glycine conjugate was isolated and purified by TLC as described above with the exception that a solvent system of 60:40:2 diethyl ether: petroleum ether: formic acid (v/v/v) was used for optimum separation.

ELISA Procedure

1. Microtiter plates were coated by adding 100 μL of coating antigen per well (0.023 mg protein per mL) and incubating for 30 min at room temperature.

2. The plate was emptied and washed once with PBS-Tween (200 μL per well).

3. Unoccupied sites on the polystyrene well surface were blocked by treating with a 5% (w/v) solution of powdered milk in PBS (200 μL per well) for 30 min at room temperature.

4. The plate was emptied and washed two times with PBS-Tween as above.

5. Diluted antiserum (1:1000) was preincubated (15 min) with herbicide standard and sample solutions. Aliquots (100 μL per well) of the preincubated mixture were transfered to the wells of the microtiter plate and incubated for 1 h at room temperature. One column of the plate received no coating and no herbicide in order to determine non-specific binding while another column received diluted antisera only to determine the maximum absorbance reading (B_o).

6. The plate was emptied and washed two times with PBS-Tween.

7. Goat antirabbit phosphatase conjugate diluted in PBS (1:5,000,000) was added (100 µL per well) to the plate. The plate was incubated for 30 min at room temperature.

8. The plate was emptied and washed two times with PBS-Tween.

9. Substrate (1 tablet per 5 mL of diethanolamine buffer) was added to the plate (100 µL per well). Color was allowed to develop for 1 h or until a reading of 0.6 to 0.8 AU was obtained.

10. Absorbance of each well was measured at 405 nm. Absorbance of the standards corrected for non-specific binding was divided by B_0 (also corrected for non-specific binding). This value was plotted against the log of herbicide concentration (ng/mL) to construct a standard curve. Concentrations of unknowns were calculated on the basis of the standard curve.

RIA Procedure. The following RIA procedure is a modified version of that described by Weiler et al. (1986).

1. Into 1.5 mL microcentrifuge tubes (Fisher Scientific, Don Mills, ON) was transfered 100 µL of standard or sample. Control tubes received 100 µL of non-fortified sample solution.

2. Incubation mix (300 µL per tube) consisting of one part deionized water, one part inert serum, 12 parts PBS, and sufficient radiolabel to yield 10,000 cpm per assay was added to each tube.

3. Antisera diluted in PBS (1:100 for 2,4-D antisera, 1:600 for picloram antisera) was added to the tubes (100 µL per tube). One set of control tubes did not receive antisera for determination of non-specific binding and a second set of control tubes received antisera only for maximun binding of radiolabel (B_0).

4. The contents of the tubes were mixed thoroughly on a vortex mixer followed by a 2 h incubation at 4°C.

5. The antibody-bound radiolabel fraction was precipitated by adding 0.5 mL of a 90% saturated $(NH_4)_2SO_4$ solution, mixing, and incubating for 1 h at 4°C.

6. The precipitate was centrifuged (12,000 x g) for 5 min and the supernatant was discarded. The pellet was washed once with a 0.5 mL portion of a 50% saturated $(NH_4)_2SO_4$ solution. The tubes were re-centrifuged and the supernatant discarded. The pellet was dissolved in two 300 µL aliquots of deionized water which were transfered to 6 mL scintillation vials. Each vial received 4 mL of scintillation cocktail (Aquasol 2).

7. The scintillation vials were assayed for radioactivity. All results were corrected for non-specific binding. Values for standards were divided by B_0 and were plotted against the log of the herbicide concentration (ng/mL). The quantity of the herbicide in the unknown sample was calculated based on the standard curve.

RESULTS AND DISCUSSION

A linear relation between the log of 2,4-D concentration and relative absorbance (B/B_0) was found in the range of 100 to 10,000 ng/mL for the indirect ELISA procedure (Figure 1A). A similar relationship was shown between 50 and 10,000 ng/mL of 2,4-D (Figure 1B) and between 50 and 5000 ng/mL of picloram (Figure 2) for the RIA procedures. Statistical analyses showed that for each method, the slope of the standard curve remained constant between experimental runs while the elevation of the line was subject to small variations. The coefficient of variation (c.v.) within a run was 7% or less for 2,4-D determined by the indirect ELISA method; 9% or less for 2,4-D by the RIA method; and 3% or less for picloram determined by the RIA method.
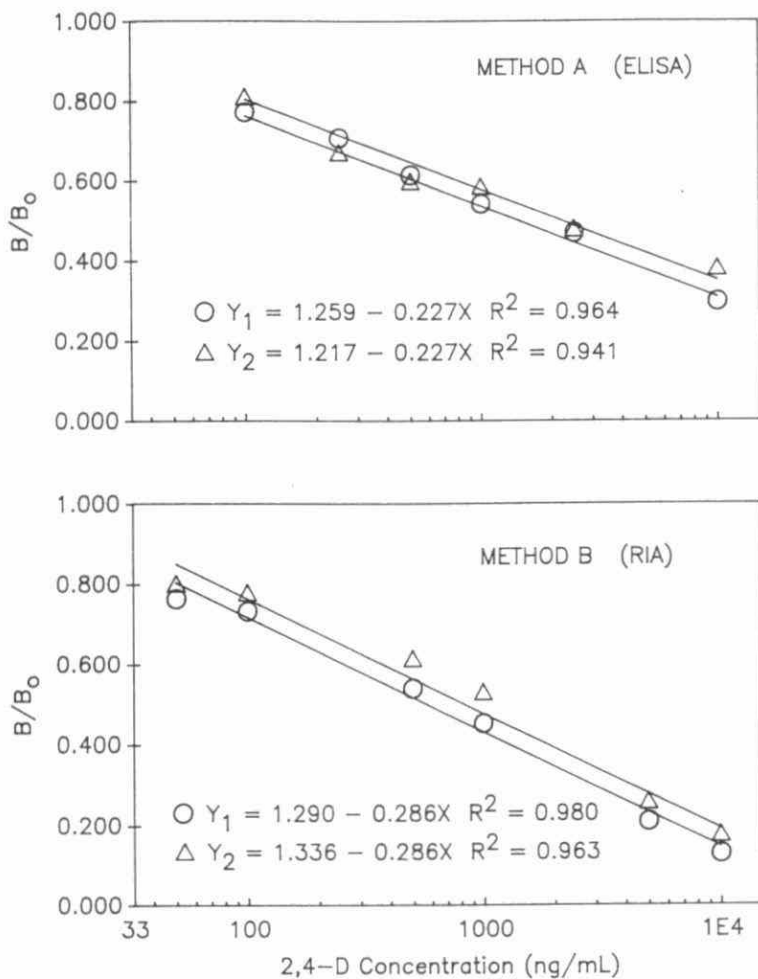
Figure 1. Standard curves for duplicate runs for the determination of 2,4-D by Method A (ELISA) and Method B (RIA). Each point represents the mean of four or five determinations.
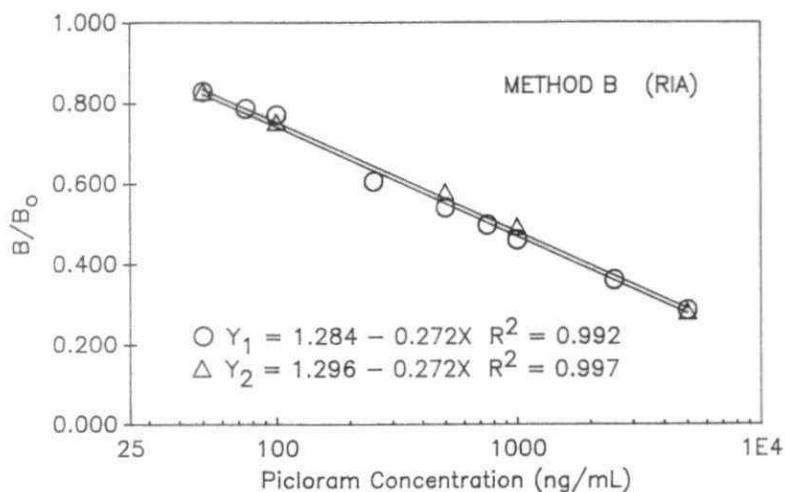
255

Figure 2. Standard curves for duplicate runs for the determination of picloram by RIA. Each point represents the mean of four or five determinations.

Table I. Recovery of analyte from river water and human urine samples as determined by RIA or indirect ELISA.

Method	Analyte	Amount of analyte added, μg/mL	Recovery[a] River water	Human urine
RIA	2,4-D	0.25	0.21 ± 0.04 (4)	0.25 ± 0.01 (8)
		2.50	2.35 ± 0.04 (4)	2.65 ± 0.15 (7)
ELISA	2,4-D	0.25	0.29 ± 0.06 (2)	0.25 ± 0.03 (9)
		0.75	ND[b]	0.90 ± 0.13 (7)
		2.50	2.44 ± 0.92 (2)	ND
RIA	picloram	0.25	0.25 ± 0.03 (6)	0.19 ± 0.04 (6)
		2.50	2.60 ± 0.19 (6)	2.22 ± 0.35 (6)

[a]Mean recovery: μg/mL ± SE (number of determinations).
[b]ND = not determined.

Recoveries from fortified river water and human urine samples determined by the immunoassays were good with mean overall recoveries varying from 82% to 110% (Table I). The range of concentrations over which 2,4-D and picloram was accurately quantitated with no sample clean-up correspond with levels found in urine in applicator exposure studies conducted by Libich et al. (1984) as well as with levels reported from environmental fate studies conducted by Thompson et al. (1984) and Hall et al. (1987). With a concentration step, such as the one described by Fleeker (1987) using disposable reverse-phase preperative chromatography columns (octadecylsilane bonded phase packing; C_{18}), the immunoassays also could be applied to well water contamination studies where a lower limit of detection is required (Frank et al., 1987).

To determine the specificity of the antisera for 2,4-D, a RIA was conducted whereby binding of the 2,4-D-[^{3}H]glycine radiolabel was inhibited with structurally similar herbicides at concentrations up to 10,000 ng/mL. The specificity of the picloram antisera was determined in a similar manner. The results indicated that 2,4,5-T, MCPA, and 2,4-DP cross-reacted with the 2,4-D antisera to some extent (Table II). The antisera was six times more specific for 2,4-D than for the

TableII. Specificity of antisera for 2,4-D or picloram compared to some structurally similar herbicides determined by the RIA method.

Antisera	Compound	Amount of compound required for 50% inhibition in binding of radiolabel, ng/mL
2,4-D	2,4-D	560
	MCPA	3,600
	2,4,5-T	5,000
	2,4-DP	10,000
	MCPP	>10,000
	dicamba	>10,000
picloram	picloram	760
	clopyralid	>10,000
	triclopyr	>10,000
	2,4-D	>10,000

strongest competitor, MCPA. None of the related herbicides tested were able to inhibit binding of the picloram radiolabel by 50%. The lack of specificity of the picloram antisera for 2,4-D is particularly important since picloram is sold commercially as a mixture with 2,4-D.

The RIA methods reported here incorporate a novel radiolabel. Herbicides labelled with ^{14}C are easily obtained but do not lend themselves to sensitive and accurate immunoassay work because of low specific activities (Hammock and Mumma, 1980). Radioimmunoassays utilizing high specific activity radiolabels such as [^{3}H]2,4-D (Knopp et al., 1985) or an [^{125}I]2,4-D derivative (Rinder and Fleeker, 1981) have given good results. Covalently linking the herbicide molecule with [^{3}H]glycine yields a radiolabel with high specific activity without the expense of purchasing a custom synthesized tritiated herbicide or the health hazards connected with iodated radiolabels.

The RIA method was found to be more reliable than the ELISA method. In practice, the RIA was a much simpler procedure requiring fewer steps to complete the assay. The formation of the antibody-antigen complex depends on a combination of weak non-covalent bonds including hydrogen bonds, electrostatic forces, Van der Waals forces, and hydrophobic bonds. Likewise, the passive binding of coating antigen to the microtiter plate well surface depends on these same forces. A successful indirect ELISA requires the formation of these weak bonds at three separate sites: i) between the plate surface and the coating antigen, ii) between the coating antigen and the antibody, and iii) between the antibody and the goat antirabbit-enzyme complex. In comparison, the RIA relies only on the formation of the antibody-antigen complex. It is our opinion that for pesticide determinations, a direct ELISA with monoclonal antibodies specific for the particular herbicide would provide a more simple and reliable assay.

The immunoassays reported here could be incorporated on a routine basis in most laboratories to serve one of two functions. The assays could be used as a rapid, inexpensive method for herbicide quantitation with no sample clean-up. Alternatively, they may be implemented as a preliminary screen to rank samples for follow-up determination by gas chromatography. In either function, the immunoassays represent savings in time, labor, and materials.

Abbreviations Used. ELISA: enzyme-linked immunosorbent assay, RIA: radioimmunoassay, NHS: *N*-hydroxysuccinimide, DCC: *N,N'*-dicyclohexylcarbodiimide, CMC: 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate, PBS: phosphate buffered saline, BSA: bovine serum albumin, RSA: rabbit serum albumin.

LITERATURE CITED

Baur, J. R.; Bovey, R. W.; Merkle, M. G. *Weed Sci.* **1972**, *20*, 309-313.

Cheung, P. Y. K.; Gee, S. J.; Hammock, B. D. In "Impact of Chemistry on Biotechnology"; Phillips, M., Shoemaker, S. P., Middlekauf, R. D., Ottenbrite, R. M., Eds.; American Chemical Society: Washington, DC, **1988**; *ACS Symp. Ser.* No 362, pp 217-229.

Fleeker, J. R. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 874-878.

Frank, B.; Clegg, B. S.; Ripley, B. D.; Braun, H. E. *Arch. Environ. Contam. Toxicol.* **1987**, *16*, 9-22.

Grover, R.; Cessna, A. J.; Muir, N. I.; Riedel, D.; Franklin, C. A.; Yoshida, K. *Arch. Environ. Contam. Toxicol.* **1986**, *15*, 677-686.

Hall, J. C.; Bowhey, C. S.; Stephenson, G. R. *Proc. 1987 Brit. Crop Prot. Conf. - Weeds.* **1987**, *2*, 593-599.

Hamaker, J. W.; Johnston, H.; Martin, R. T.; Redemann, C. T. *Science* **1963**, *141*, 363.

Hammock, B. D.; Mumma, R. O. In "Recent Advances in Pesticide Analytical Methodology"; Harvey, J., Jr., Zweig, G., Eds.; American Chemical Society: Washington, DC, **1980**; *ACS Symp. Ser.* No 136, pp 321-352.

Hoar, S. K.; Blair, A.; Holmes, F. F.; Boysen, C. D.; Robel, R. J.; Hoover, R.; Fraumeni, J. F., Jr. *J. Amer. Med. Assoc.* **1986**, *256*, 1141-1147.

Knopp, D.; Nuhn, P.; Dobberkau, H.-J. *Arch. Toxic.* **1985**, *58*, 27-32.

Libich, S.; To, J. C.; Frank, R.; Sirons, G. J. *Am. Ind. Hyg. Assoc. J.* **1984**, *45*, 56-62.

Ragab, M. T. H. *Can. J. Soil Sci.* **1975**, *55*, 55-59.

Rinder, D. F.; Fleeker, J. R. Bull. *Environ. Contam. Toxicol.* **1981**, *26*, 375-380.

Thompson, D. G.; Stephenson, G. R.; Sears, M. K. *Pestic. Sci.* **1984**, *15*, 353-360.

Van Emon, J. M.; Seiber, J. N.; Hammock, B. D. In "Bioregulators for Pest Control"; Hedin, P. A., Ed.; American Chemical Society: Washington, DC, **1985**; *ACS Symp. Ser.* No 276, pp 307-316.

Weiler, E. W.; Eberle, J.; Mertens, R.; Atzorn, R.; Feyerbrand, M.; Jourdan, P. S.; Arnscheidt, A.; Wieczorek, U. "Antisera- and Monoclonal Antibody-Based Immunoassay of Plant Hormones". In *Immunology in Plant Science*; Wang, T. L., Ed.; Cambridge University Press, Cambridge. **1986**.

SESSION D
ANALYTICAL METHODS
Oral Presentations

DERIVATIZATION OF ACIDIC ORGANIC COMPOUNDS USING PHASE TRANSFER
CATALYSIS

V.Y. Taguchi and O.W. Berg

Ontario Ministry of the Environment, Rexdale, Ontario M9W 5L1

Derivatization procedures are usually incorporated into the
analyses of acidic organic compounds such as phenols and
carboxylic acids in order to improve their chromatographic
properties. Classical organic chemistry is the basis for some
common derivatizing agents. Diazomethane has been used to
derivatize phenols and carboxylic acids to their corresponding
anisoles and methyl esters respectively. Acetic anhydride has
been used to acetylate phenols, alcohols and amines. Acetylation
relies on the nucleophilicity of the phenol and the
electrophilicity of the acetylating agent. Some phenols such as
2,4-dinitrophenol and 4,6-dinitro-o-cresol are difficult to
derivatize with acetic anhydride. This may be due to
delocalization of electron density from the phenolic oxygen into
the nitro groups via resonance stabilization. The net result may
be a poor nucleophile.

Phase transfer catalysis or extractive alkylation was
applied to phenolic compounds by Rosenfeld and Taguchi [1]. The
phase transfer agent used was tetrahexylammonium hydroxide
(THAH). The following scheme illustrates this technique.

$$ArOH \quad + \quad OH^- \ \rightleftharpoons \ ArO^- \quad + \quad H_2O \qquad (1)$$

$$ArO^-_{aq} \quad + \quad THA^+_{aq} \ \rightleftharpoons \ [THA^+/ArO^-_{aq}] \qquad (2)$$

$$[THA^+/ArO^-_{aq}] \quad \rightleftharpoons \quad [THA^+/ArO^-_{org}] \qquad (3)$$

$$[THA^+/ArO^-_{org}] \quad + \quad CH_3I \rightarrow ArOCH_3 \quad + \quad THA^+I^- \qquad (4)$$

The phenol (ArOH) is ionized in an alkaline solution (step 1). The phenol anion forms an ion pair with a tetraalkylammonium cation (THA$^+$) (step 2). This ion pair is soluble in both the aqueous and the organic phases and is partitioned between them (step 3). If the organic phase contains an alkylating agent such as methyl iodide, irreversible alkylation occurs in this phase (step 4). This shifts the equilibrium towards the alkylated derivative. This process relies on formation an ion pair of the analyte with the phase transfer agent. The speed of derivative formation and the yield of the derivative are dependent on the efficiency of the extraction and the rate of reaction of the ion pair with the alkylating agent. The efficiency of extraction of the anion of the analyte is determined by the properties and concentrations of the ions of the ion pair, the lipophilicity of the ion pair and the properties of the solvent. The most frequently used phase transfer agents are the tetrabutyl-, tetrapentyl- and tetrahexylammonium hydroxides. In general, the pH of the aqueous phase should be at least 2 units higher than the pKa value of the acidic analyte. The organic solvents are

generally aprotic such as dichloromethane or toluene although some alcohols have been used.

Phase transfer catalysis has been applied to a series of substituted phenols (alkylated, chlorinated and nitrated). Initial experiments have shown that methylation to the corresponding anisoles occurs within 1 hour at room temperature for most of the phenols studied. No product was obtained with 4,6-dinitro-o-cresol. This may be due to lack of formation of the ion pair because of steric hindrance. This may be a limiting factor in the applicability of the technique.

One of the problems associated with phase transfer catalysis is elimination of the excess and reacted ion pair agent. This was accomplished using a short column of Florisil. The anisole derivatives were eluted with dichloromethane while the ion pair agent remained on the column. When an insufficient amount of Florisil was used, excess reagent was isolated with the products. In a GC-MS equipped with an on-column injector, the excess THAH underwent a Hoffmann degradation to form trihexylamine which appeared as a broad peak in the chromatogram.

Further experiments are underway to optimize conditions for environmentally significant phenols.

1. J.M. Rosenfeld and V.Y. Taguchi, Anal. Chem., 48, 726 (1976).

NEW CHEMICAL IONIZATION REAGENTS DIRECTED TOWARD MASS
SPECTROMETRIC ANALYSIS OF TRACE ORGANICS

T. B. McMahon, K. Froese and C. E. Allison
Department of Chemistry and Guelph-Waterloo Centre
for Graduate Work in Chemistry
University of Waterloo
Waterloo, Ontario N2L 3G1

Although electron impact ionization is by far the most commonly used ionization technique in analytical mass spectrometry, chemical ionization methods offer unique advantages in many specialized circumstances. Chemical ionization simply makes use of reagent ions generated by ion-molecule reactions in a reagent gas to react with analyte introduced into the CI ion source. For example, the simplest and most frequently used CI technique is CH_4 proton transfer CI in which the ions CH_5^+ and $C_2H_5^+$, generated by reaction of the electron impact produced ions CH_3^+ and CH_4^+ with CH_4 (eqns (1) and (2)) act as super acids in the gas phase toward the analyte, A (eqn(3)).

$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2 \tag{1}$$

$$CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3 \tag{2}$$

$$CH_5^+(C_2H_5^+) + A \rightarrow AH^+ + CH_4(C_2H_4) \tag{3}$$

Working from a broad base of understanding of the energetics and mechanisms of gas phase ion molecule reactions it is possible to exploit these aspects of the dynamics of reactions to tailor chemical ionization reagents to fill specific needs. For example, in proton transfer CI the amount of energy deposited in the conjugate acid of the analyte will determine the extent of fragmentation (Scheme 1). It is possible to control the amount of energy deposited in AH^+ and

265

$$RH^+ + A \rightarrow (AH^+)^{\bullet} + R$$

$$F_1^+ \quad F_2^+ \quad F_3^+$$

$$+N_1 \quad +N_2 \quad +N_3$$
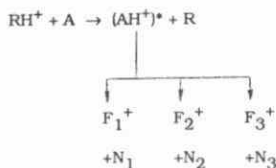
(SCHEME 1)

hence control the extent of fragmentation by using reagent ions derived from compounds R of varying gas phase basicity.

Negative chemical ionization (or ion-molecule reactions involving anions) have received widespread attention recently as a potential means of analysis of compounds of environmental interest, particularly PAH's.   However, the use of negative ion EI or CI techniques with chlorinated organics is hampered by the relatively weak C-Cl bond strength and the high electron affinity of chlorine which leads to a considerable degree of dissociative ionization, eqn(4). Thus in such ionization events all information regarding the analyte ACl is lost since the

$$R^- + ACl \rightarrow (R + A) + Cl^- \tag{4}$$

ionization occurs predominantly as Cl^-. This result can be regarded as being due to the chemical activation of the intermediate ion molecule complex $(RACl^-)^*$ which is sufficient to induce unimolecular decomposition. In order to limit the extent of unimolecular decomposition it is again worthwhile to limit the extent of chemical activation of the $(RACl^-)$ intermediate which can be done by a judicious choice of reagent ion R^-.

Chemical ionization experiments are most frequently performed in relatively gas tight ion sources in conjuction with either magnetic sector or quadrupole mass spectrometric detection. At the pressure employed (~1 torr) with ion residences times of several μsec the average reagent ion will have undergone at least several hundred collisions. Since it is a normal feature of ion molecule reactions that they occur with near unit collision efficiency this means that all or nearly

all of the reagent ion intensity can be converted to analyte ion intensity under appropriate conditions.

All of the experiments done in conjuction with this project were carried out with Fourier Transform Ion Cyclotron Resonance Spectrometry (FTICR). This technique accomplishes chemical ionization at pressures as low as 10^{-9} torr by trapping ions for extremely long periods of time. Most typically in analytical situations pressures of 10^{-6} torr are used with trapping times on the order of 1 sec which allows roughly the same number of collisions to occur as in a conventional CI system. The only differences between the FTICR method and the usual CI system is that at the low pressures of the FTICR termolecular clustering reactions are avoided.

Ethylchloroformate proved to be by far the most promising of the reagents investigated. The sequences of reactions, eqns(5)-(7) readily leads to production of chloride ion adducts. A

$$ClCO_2C_2H_5 \rightarrow Cl\text{-} + CO_2C_2H_5 \tag{5}$$

$$Cl^- + ClCO_2C_2H_5 \rightarrow ClCO_2^- + C_2H_5Cl \tag{6}$$

$$ClCO_2^- + A \rightarrow ACl^- + CO_2 \tag{7}$$

competing reaction, eqn(8) potentially leads to a decrease in intensity of ACl^-, however, for most substrates, A, eqn(9), also occurs, leading again to ACl^-. The excellent signal intensities

$$ClCO_2^- + ClCO_2C_2H_5 \rightarrow Cl_2CO_2C_2H_5^- + CO_2 \tag{8}$$

$$Cl_2CO_2C_2H_5^- + A \rightarrow ACl^- + ClCO_2C_2H_5 \tag{9}$$

achieved with this reagent system and the facile transfer of Cl^- from CO_2 to a wide variety of compounds led us to investigate extensively the potential of this compound as a Cl^- transfer CI reagent.

This reagent system appears to be applicable to a wide variety of compounds including PAH's and chlorinated aromatics. Representative spectra are shown in **Figure 1**.

The results of FTICR experiments indicate that chloride attachment CI using $ClCO_2C_2H_5$ as a chemical ionization reagent can potentially yield excellent intensities in

quasimolecular ions of a single molecular formula. The method thus has promise in terms of sensitivity for CIMS analysis of compounds ranging from PAH's to dioxins.

DP3
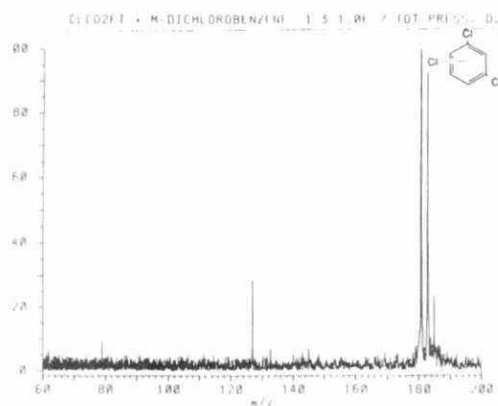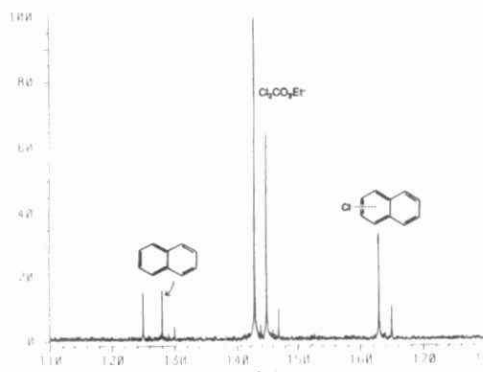
An Interrupted Segmented Flow Stream Microwave Solid Sample Decomposition System for ICP-AES

E.D. Salin and Baoquin Liu
Department of Chemistry
McGill University
801 Sherbrooke St. W.
Montreal, P.Q., Canada
H3A 2K6
phone 514 398 6236

Microwave aided digestion offers significant speed advantages over conventional atmospheric or bomb digestion techniques for certain sample types (1-4). It is our hope to develop a flowing stream microwave system suitable to the digestion of solid samples. It is our expectation that such a system would provide many of the throughput advantages enjoyed by segmented and flow injection systems. In addition, automated handling of liquids should minimize the fume hazards which can result when using the more powerful reagents required for some difficult digestions.

Sample, as a 0.2 g slurry in 25 ml is aspirated into tubing where it is mixed at a "T" junction with 25 ml of 0.2 M reagent grade nitric acid. During this process Valves 1 and 2 are open. A conventional "kitchen" type microwave oven has been modified by the addition of two 3/8 in holes for the entrance and exit of the tubing and the addition of an exhaust fan to aid in the removal of hot air. While this oven has operated properly, we encourage researchers to buy industrial calibre microwave ovens for several reasons, (1) they can tolerate higher internal temperatures and (2) they allow considerably more flexibility in their heating cycles.

Initially, Valves 1 and 2 are open. The slurry/acid solution is pumped into the microwave and Valves 1 and 2 are closed. The solution is then subjected to the microwave radiation. At the completion of the exposure, the system is allowed to cool approximately 1 minute. Valve 2 is then opened to relieve pressure. Any effluent is collected in the collection vessel. Then Valve 1 is opened and the tubes are removed from the slurry solution and the acid reservoir. The sample plug is followed by an air bubble. The system can be cleaned by an acid plug or rinsed with a water plug or acid plug.

The demonstration problem undertaken is one common to one of our undergraduate laboratories, the digestion of Cu Ore using nitric acid. For comparison purposes, we have carried out experiments using the apparatus illustrated in Figure 1 and using a traditional atmospheric hot plate digestion procedure. The sample was in a 325 mesh (44 micron) particle size format. The sample was used in exactly the same format with the same reagents in both experiments.

The results are presented succinctly in Figure 2. Compared with conventional atmospheric digestion conditions, the stream microwave system is an order of magnitude faster. Both the temperature and the pressure are measurably elevated over those available at atmospheric pressure. These experiments were designed to test the effectiveness of the energy coupling into tubing of the dimensions employed. They were obviously a success in this regard. We hope to develop a continuous flow system for rapid continuous digestion of solid samples.

1.   Smith, F; Cousins, B.; Bozic, J.; Flora, W. *Anal.Chim.Acta* **1985**
2.   Lamothe, Paul J.;Fries, Terry L.;Consul, Jerry J. *Anal.Chem.* **1986**, *58*, 1881.
3.   Alvarado,Jose;Leon, Luis, E.;Lopez, Francisco;Lima; Carlos *JAAS*, **1988**, *3*, 135.
4.   Kingston, H.M.; Jassie, L.B. *Anal.Chem.* **1986** *58* 2534.

Microwave Solid Stream Digestion Apparatus
First Experimental Configuration
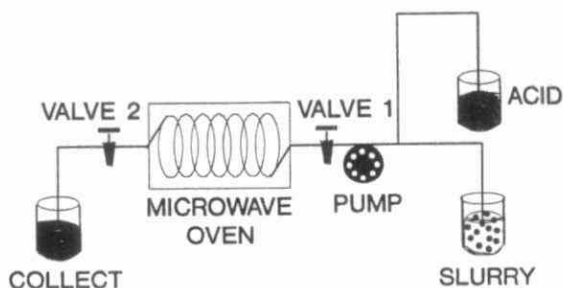
Figure 1

Digestion Times
0.2 g of Cu Ore

Figure 2

DP4
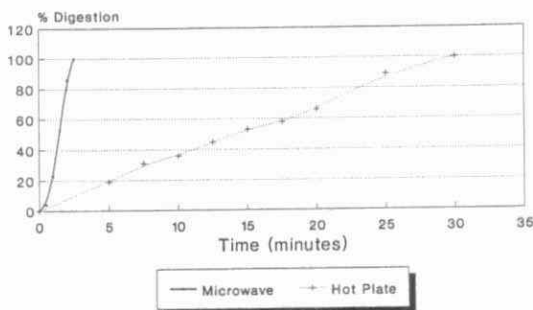
SOLID PHASE EXTRACTION OF PAH's FROM DRINKING WATER AND THE ANALYSIS OF CHLOROPHENOLS AND PHENOXY-ACID HERBICIDES IN WATER.

J.M. Craig, B. Derry and W.G. Craig, Paracel Laboratories Ltd., 8-17 Grenfell Cr., Nepean, ON, K2G OG3; C.D. Hall, Laboratory Services Branch, MOE

The current procedures used to analyze polyaromatic hydrocarbons (PAHs) in drinking water and chlorophenols and phenoxyacid herbicides in environmental aquatic samples involve liquid/liquid extraction. In the case of the PAHs, the aqueous sample is extracted with methylene chloride and subsequent analysis of the organic extract involves the use of high pressure liquid chromatography (HPLC) with a fluorescence detector(1). Methylene chloride is also used to extract the chlorophenols and phenoxyacid herbicides after acidification of the aqueous sample. Prior to quantification, this extract is alkylated using diazomethane and cleaned-up on florisil. Quantitation is by gas chromatography (GC) with dual electron capture detectors(2).

Although these methods are well established, the potential exists to simplify these procedures by the use of solid phase extraction (SPE) for both the extraction and clean-up steps. The advantages of SPE are:
  1) increased throughput,
  2) increased consistency of the analytical results,
  3) decreased costs of shipping and storage, and
  4) being amenable to automation with robotics.

Previous work demonstrated the feasibility of using SPE to recover chlorophenols and phenoxyacids with C-18 columns(3). This work has been extended by an in-depth examination of factors affecting the recoveries of these compounds with particular attention being paid to the reduction of compounds which co-elute and are detected by the electron capture detector. Data attests to the successful use of SPE in field studies. A feasibility study has been initiated for the use of SPE for the analyses of PAHs in aquatic samples.

In order to facilitate the analysis of chlorophenols and phenoxyacids they are derivatized using diazomethane(DAM) to form the methyl derivative. However, both DAM and its precursor, N,N-dimethylnitrosourea (guanidine) are suspected carcinogens and represent a health hazard to laboratory personnel. Alkylation of carboxylic acids and phenols can be achieved by a variety of reactions that are less hazardous. These include:
  1) on-column alkylation with Methelute,
  2) extractive alkylation with tetrabutylammonium hydroxide,
  3) methyl iodide/base in acetone.

The potential of these three procedures to replace the use of diazomethane has been investigated.

271

Findings:

A: Solid Phase Extraction

1) The consistency of batches from five different lots of
J.T.Baker C-18 SPE columns (100 mg) was examined first. The
recoveries of the tri-, tetra- and pentachlorophenols and of
dicamba, 2,4-dichlorophenoxypropionic acid, 2,4-D, 2,4,5-T,
silvex, 2,4-DB and picloram were determined. Values for the
blanks were also determined. The variation between lots was found
to be comparable to the variations within lots.

2) Larger SPE columns, 500 mg and 1 g, gave much faster flow
rates and allowed more sample volume to be passed before becoming
blocked. However, these larger columns gave extremely high levels
of interferences detectable by GC/ECD necessitating treatment of
the columns prior to use.

3) The volumes of methanol and water used to condition the
columns was not critical nor were sample flow rates at practical
levels.

4) Very fast elution flow rates were found to reduced the
recoveries and also decrease the reproducibility.

5) The effect both of introducing various wash solvents (before
and after extraction of the sample) and of varying the eluting
solvent composition on the level of interferences is being
investigated.

6) The levels of interferences obtained using SPE for PAHs and
the influence of the solid phase and eluting solvent on their
recoveries was studied.

B: Derivatization of chlorophenols and phenoxy-acids

1) Methyl iodide and sodium carbonate:

The reaction was studied with a range of solvents,
temperatures, reaction times and concentrations of methyl iodide.
The optimum conditions involved heating methyl iodide (20
microlitres) and anhydrous sodium carbonate (5 mg) together with
the parent acids and phenols in acetone (1 ml) in a capped vial
at 55°C for 1 hour. Separation of the liquid phase, evaporation
of the bulk of the solvent, followed by dilution with hexane
furnished the derivatized analytes ready for injection onto the
GC. Reproducible results, comparable to those obtained using
diazomethane, were obtained over a wide range of analyte
concentrations.

2) On-column Derivatization:

Chlorophenols and phenoxyacids were co-injected with
Methelute (trimethylanilinium hydroxide) onto the GC columns

using a variety of Methelute concentrations, solvents and injection temperatures. This technique gave higher levels of interferences and generally the reactions were incomplete as compared to diazomethane. Picloram gave quite low yields of the methylated product.

3) Extractive Alkylation:

This technique was investigated using both tetrabutylammonium hydroxide (TBAH) and trimethylanilinium hydroxide as the counter-ion and with methyl iodide. The reaction was studied for a range of temperatures, reaction times and concentrations of methyl iodide and analytes. TBAH was found to be the most effective counter-ion. Although methylation by this method was more effective than on-column derivatization, it was less reproducible and gave generally lower yields than either diazomethane or methyl iodide/sodium carbonate, especially for picloram.

Conclusions:

1-The study on the use of SPE for the extraction of PAHs from water is continuing.

2-Replacement of diazomethane by methyl iodide/base in the derivatization step for the analysis of chlorophenols and phenoxyacids will be evaluated in field samples.

3-Attempts will be made to combine extraction with the derivatization step in the analysis of chlorophenols and phenoxy acids.

References:

1. MOE Method HPLC/L-X3HIDI. The determination of polynuclear aromatic hydrocarbons in surface water, domestic water and industrial wastes by HPLC.

2. MOE Method PWACP/PA. The determination of chlorophenols & phenoxy acids in effluents and water by GC-ECD.

3. Report No 2. MOE contract A94836. March 26, 1987.

DP5

"AUTOMATED WATER SAMPLER FOR DIOXIN DETECTION AT PPQ LEVELS"
B. R. Hollebone, C. Shewchuk* and L. Brownlee, Chemistry
Department, Carleton University, Ottawa, Ontario; S. Suter and H.
Tosine, Laboratory Services Branch, MOE; R. Hunsinger and M. Uza,
Water Resources Branch, MOE.

The Ministry of the Environment is responsible for many monitoring programs for organic contaminants. In particular, there is a need to detect and quantify accurately the highly toxic polychlorinated dibenzo-p-dioxins (PCDDs) and chlorinated dibenzofurans (PCDFs) in the environment. Special attention is directed at determining the exposure of the population to these substances in drinking water. The current method of analysis for these compounds in drinking water is by liquid-liquid extraction of 10 L grab samples. The public demand for more information on water quality requires lowering the detection limit to 1.0 ppq while increasing the number of samples and maintaining high standards of Quality Assurance—Quality Control.

The most effective way to meet these conflicting demands is automated preconcentration at the test site. A safe, portable sampling unit was required because existing liquid solvent extractors cannot be used in a water treatment plant. One example is the APLE unit which uses 8 L of solvent for a 200 L batch of water. This unit was found to be too bulky and cumbersome to fit the criteria of a portable sampling unit and, the volume of solvent is not appropriate from a safety pint of view. Other liquid-liquid extractors do not follow safety restrictions since they involve discharge of dichloromethane.

275

In order to automate the preconcentration process the construction of an instrument was required in order to provide more acceptable collection techniques. Studies of liquid-solid extraction of organics from water shown that the technique could be used in a sampling unit. It is essential that in addition to the column, a filtering system be installed since raw and treated water which may contain particulate matter, could be analyzed. Two generations of automatic preconcentration samplers (APS) have been built in our laboratory to comply with the necessary criteria. The first prototype, Mark I APS, assisted in determining the important features for the Mark II, permitting the establishment of specific requirements for a safe, convenient analytical system.

The Mark II APS was designed to be rugged and portable for use at a wide variety of sampling sites. The preconcentration vessels (filters and columns) were made detachable so they may be returned to a central laboratory for analysis. Many safety features were incorporated to ensure safe use in a water treatment plant.

In the Mark II sampling unit a water reservoir was incorporated into which the raw or treated water sample is pumped. It provides a physical air break in the sample stream between the water and the sampler to prevent any black flow to the source. The sampler is also capable of providing a continuous injection and mixing of standards throughout the sampling period. A standard injection assembly consisting of a reservoir and precision pump can deliver the standard solution as a constant

proportion of the sample flow rate. This dynamic quality control of sampler performance during a sample run provides a valid reference for quantitation of the analyte as a function of time and matrix of the sample.

The preconcentration process in the sampler system provides a dual function; first to filter particulate from the sample stream and then to adsorb the remaining water soluble target compounds on the column for subsequent analysis. The filter system is based on setting the distinction between dissolved and insoluble particles at 0.45μm. The duplicate analytical systems each consist of 2 tubular filters placed in series to accommodate 50-100L of raw water. The adsorption technology in the present sampler utilizes XAD-2 resin for which sampling conditions and elution and detection procedures have been established to permit recovery and analysis of dioxins at the ppq level.

A schematic of water flow through the sampler is illustrated in figure 1. The overall design and placement of components are shown in figures 2 and 3. The water sample from a pressurized source enters through the "water in" port located at the back of the sampler into the water reservoir. The water is drawn through a mixing chamber, where the incoming water will be mixed with an internal standard, and is then split equally to provide duplicate samples. Each sample stream passes through a filter assembly, a high precision gear pump, an air release valve, and then a column. The air release valve is essential for removing air accumulated on the filter, preventing it from entering the column

277

and monitoring components. One sampling apparatus is located on each side of the sampler. The pumps are responsible for regulation of flow rate. The water flow and pressure of each sample stream is monitored by mechanical flow meters and pressure gauge to ensure identical sampling conditions. A pressure switch, located between the filter and column, is connected to a batch controller and can terminate a sample run if the pressure build-up from the columns occurs, or if low pressure due to cavitation from a blocked filter occurs. After passing through the columns, the two sampling streams join to pass the main electronic flow sensor which monitors the total water flow of the system. The flow sensor sends a signal to a digital flow meter and chart recorder for a readout and hard copy of the flow rate, and to the batch controller which records the total sample volume. A charcoal filter is placed prior to sample output to remove any residual organics. An analytical bypass controlled by three-way diverter valves diverts the water sample from the mixer to the charcoal filter. This feature flushes any residual water out of the reservoir and replaces it with a fresh water sample before initiating another sample run.

FIGURE 1

CHART RECORDER

SIGNAL CONVERTER

DIGITAL FLOW METER

EXHAUST

NCV

CHARCOAL FILTER

FS

ANALYTICAL BYPASS

COLUMNS

PS

ARV

PG

PS

ARV

PG

PUMPS FLOW METERS

FILTERS

DATA CONTROLLER

MICRO PROCESSOR

SPIKE OPTION

MIXER

CHARCOAL FILTER

AIR IN/OUT

NCV

CV

WATER RESERVOIR

NCV

CV

WATER IN

SCHEMATIC OF MARK II APS

---- ELECTRICAL CONNECTION
PG   PRESSURE GAUGE
ARV   AIR RELEASE VALVE
PS   PRESSURE SWITCH
FS   FLOW SENSOR
CV   CHECK VALVE
NCV   NORMALLY CLOSED VALVE

279

RIGHT SIDE VIEW

FIGURE 2

LEFT SIDE VIEW

FIGURE 3
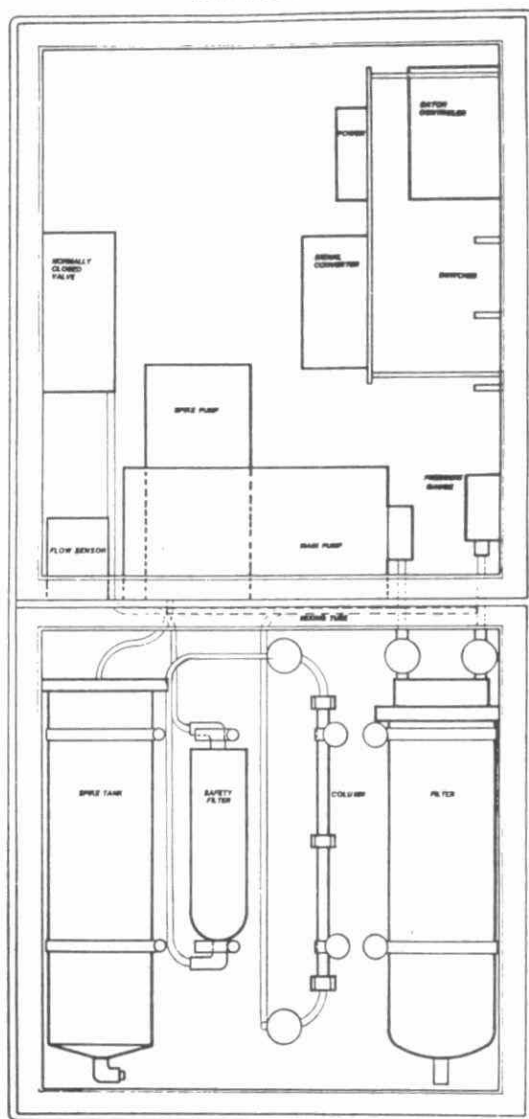
DP6

AUTOMATED HPLC METHOD FOR LOW LEVEL POLYNUCLEAR AROMATIC HYDROCARBON (PAH) ANALYSIS OF DRINKING WATERS.
P.W. Crozier and C.D. Hall, Ministry of the Environment, Laboratory Services Branch, Rexdale, Ontario, M9W 5L1

Polynuclear Aromatic Hydrocarbons are homologs of benzene in which three or more aromatic rings are joined in various configurations. Generally, they are characterized as being high melting point/high boiling point solids with low vapour pressures at ambient temperature and atmospheric pressure[1].   PAHs are relatively hydrophobic compounds which do not readily dissolve in water or water miscible solvents at high concentrations.   PAHs are formed during heating, burning and incinerating processes involving carbonaceous materials. In nature PAHs are produced by combustion of organic matter under conditions of insufficient oxygen and/or low combustion temperature.   Industrial PAH production is associated with coal/oil/gas burning power plants and combustion engines as well as steel manufacturing and petrochemical industries[2]. PAHs are major components of asphalt, coal tar and soot. Several members of this chemical class have been shown to be carcinogenic to animals and several are suspected carcinogens for man[3]. PAHs are of environmental concern because of their natural abundance, bioconcentration in fatty tissues, bioaccumulation along the food chain and carcinogenic activity.

PAHs have been extensively studied in stack gases, polluted air and food products[4,5].   More emphasis is being placed on the analysis of PAHs in drinking water due to their discovery in monitoring programs at select locations in Ontario. The implementation and expansion of sampling programs such as the Drinking Water Surveillance program (DWSP) and Municipal Industrial Strategy for Abatement (MISA) has necessitated the development of automated low level analytical techniques. A routine automated low level high performance liquid chromatography (HPLC) method was developed to analyse drinking waters for 17 individual PAHs with the most important ones for monitoring purposes being fluoranthene , benzo(b)fluoranthene,   benzo(k)fluoranthene,   benzo(a)pyrene, benzo(ghi)perylene and indeno(123cd)pyrene. The World Health Organization (WHO) states the concentrations of these six representative PAHs should not exceed 200ng/L in drinking water[6].   This new routine analytical method increases production and data quality, and reduces detection limits and data interpretation time.

The hydrophobic nature of the PAHs necessitated individual standard stock solutions be made up using 5% benzene in methanol at concentrations between 10 ug/mL and 100 ug/mL depending on the individual PAH's water solubility. These concentrations ensured complete solute dissolution and eliminated solute precipitation from solution upon refrigerated storage.

Wet chemical sample preparation involves liquid/liquid extraction followed by a three stage concentration and solvent exchange. Samples are extracted three times with dichloromethane using a roller apparatus and the subsequent extract dried through sodium sulphate. Extracts are then evaporated to dryness using rotovap apparatus/vortex evaporator/nitrogen blow-down and the solute resuspended in acetonitrile for instrumental analysis.

Instrumental analysis is done by reversed phase HPLC. Individual components are separated using a Vydac reversed phase C18 column and solvent gradient program. A programmable Perkin Elmer LS4 fluorescence detector is used to achieve the necessary detection limits to monitor PAHs at/or below World Health Organization drinking water guidelines.

Instrument retention times were consistent over time with coefficients of variation <0.3%. Height quantitation results were slightly better than area quantitation results with coefficients of variation approximately 5%. Fluorescence detector linearity was limited to a range of approximately 10E2 with some components such as anthracene and benzo(k)fluoranthene being less. Detector linearity ranges did however increase as the fluorescence source lamp aged and the detector became less sensitive.

Method spikes between 0.5 ng/L and 1000 ng/L were analysed to establish recoveries and reproducibility. High level spikes were diluted to established detector linearity ranges for proper quantitation. Method recoveries were comparable at all spike levels tested. Average method recoveries were > 75% for most PAHs with the exception of anthracene, benzo(a)pyrene and dimethylbenzo(a)anthracene. Anthracene losses are a direct result of evaporation during extract concentration. Benzo(a)pyrene and dimethylbenzo(a)anthracene losses appear to be related to the water

284

extraction stages of the method rather than evaporation steps. Method detection limits range from 1 ng/L for anthracene and benzo(k)fluoranthene to 50 ng/L for benzo(e)pyrene and chrysene. The detection limit for benzo(a)pyrene is 5 ng/L which is below the World Health Organization drinking water guideline of 10 ng/L.

Several areas for method modification and improvement are scheduled for investigation. Lower detection limits for some components and reduced analysis time may be achieved by further wavelength switching and gradient/flow modifications respectively. New extraction/ concentration techniques (eg. Goulden Evaporator) could increase recoveries and analytical precision by limiting extract handling procedures. The addition of environmentally significant PAH derivatives such as nitroPAHs to the scan would enhance the Drinking Water Organics Section monitoring capabilities.

[1] M.L. Lee et al., Analytical Chemistry of Polycyclic Aromatic Compounds, Academic Press, Toronto Canada, 1981, 462 p.
[2] A. Bjorseth et al., PAHs in Work Atmospheres: Occurrence and Determination, CRC Press, Boca Raton Florida, 1986, 169 p.
[3] G. Grimmer, Environmental Carcinogens: Polynuclear Aromatic Hydrocarbons, CRC Press, Boca Raton Florida, 1983.
[4] S.A. Wise et al., Characterization of PAHs from Two Standard Reference Material Air Particulate Samples, Analytical Chemistry, Vol. 58, 1986, p3067-3077.
[5] J. Maga, PAH Composition of Mesquite Smoke and Grilled Beef, Journal of Agriculture and Food Chemistry, Vol. 34, 1986, p249-251.
[6] WHO, Guidelines for Drinking Water Quality Volume 1 – Recommendations, World Health Organization Publications, Geneva Switzerland, 1984, 130 p.

SUPERCRITICAL FLUID EXTRACTION OF TRACE
ORGANICS FROM SOLID MATRICES

R.C. Burk, P. Kruus and R.H. Wightman
Department of Chemistry, Carleton University
Ottawa, Ontario K1S 5B6, Canada

G. Crawford and I.Ahmad, Ministry of the Environment,
Laboratory Services Branch, Trace Organics Section
125 Resources Road, Rexdale, Ontario M9W 5L1, Canada

Trace residue analysis of organic compounds in environmental solids is comprised of two steps; sample preparation and instrumental analysis. An autosampler can provide dedicated automation for instrumental analysis. However, the sample preparation, which involves sample extraction and purification is often the most time consuming and labor intensive operation in the analytical procedure. The extraction normally requires expensive organic solvents, is associated with human exposure to toxic chemicals, and is often not selective, a fact which necessitates the cleanup step. Supercritical fluids have shown promise of overcoming some of these difficulties in sample preparation.

A supercritical fluid is a fluid at a temperature and pressure above its critical values. Under these conditions, some fluids have properties which render them excellent solvents, even for solutes which are not soluble in them under ambient conditions. The advantages of using supercritical fluids for extraction stem mainly from the variation of density of a fluid with pressure near its critical point. Since the ability of a solvent to dissolve a solute increases with the density of the solvent, supercritical fluids offer the advantage over liquid solvents that solubilities can be varied by varying the pressure. The transport properties of a supercritical fluid are intermediate between those of the corresponding gas and liquid. Viscosities are considerably lower than those of the corresponding liquids, and diffusion coefficients are considerably higher. The kinetics of extraction of solutes from solid matrices are thus faster in supercritical fluids than in liquids. The variation of solubility with pressure in a supercritical fluid is significantly different from one solute to another. Thus, by choosing the appropriate conditions, it may be possible to selectively extract a particular solute, or class of solutes. This would preclude the need for the sample cleanup step described above. The application of supercritical fluids in the selective extraction of aromatics/alkanes, and polycyclic aromatic hydrocarbons/polychlorinated biphenyls has been documented in the literature [1,2].

Carbon dioxide is the primary solvent used in the supercritical fluid extraction of organics because its critical point is easily attained in the laboratory, and because it is not toxic. There has been considerable interest recently in the use of two component supercritical solvents. Again, the primary solvent used is carbon dioxide, but the second solvent (the co–solvent) is usually an organic liquid. The role of the co–solvent is to increase the solubility or selectivity of a particular solute or class of solutes. Thus, in the extraction of organics from soils [1], the proportion of aromatics in the extract increased as the co–solvent was changed from a less polar to a more polar one. Methanol is a popular choice for co–solvent, both in supercritical fluid extraction and in supercritical fluid chromatography.

The supercritical fluid extraction apparatus was built in the laboratory. It consists of a pump, a pressure guage, an extraction cell with an internal volume of approximately 0.3 mL, and a fused silica capillary restrictor (id 20 µm). Carbon dioxide is used as the solvent. The extract is carried from the extraction cell through the capillary restrictor into a tube containing a small amount of liquid organic solvent (e.g. dichloromethane), where the carbon dioxide vaporizes, leaving the extracted compounds in the liquid solvent. The extract is then analyzed by gas chromatography.

The results of our investigations demonstrate that the supercritical fluid extraction technique using carbon dioxide yields rapid and quantitative recovery of polycyclic aromatic hydrocarbons from various environmental solids. The work is progressing to establish conditions to selectively extract various classes of organic compounds. The use of other pure solvents, as well as binary solvents will be studied. Other variables to be investigated are temperature, pressure, method of contacting the solid with the fluid, time of contact, and solvent flow rate.

REFERENCES
1. Schnitzer,M.; Preston,C.M. "Supercritical Gas Extraction of a Soil with Solvents of Increasing Polarity" Soil Sci.Soc.Am.J. 1987,51(3),639

2. Hawthorne,S.B.; Miller,D.J. "Directly Coupled Supercritical Fluid Extraction–Gas Chromatographic Analysis of Polycyclic Aromatic Hydrocarbons and Polychlorinated Biphenyls From Environmental Solids" J.Chromatogr. 1987,403,63

DP8

AUTOMATED SAMPLE INTRODUCTION AND PRE-TREATMENT WITH FLCW
INJECTION-ICP-ES. J. F. Hopper*, F. Mo, and D. W Boomer,
Laboratory Services Branch, Ontario Ministry of the
Environment, Box 213, Rexdale, Ontario, M9W 5L1

EXTENDED ABSTRACT NOT AVAILABLE

The basic principle of flow injection is quite simple. A discrete volume of liquid sample is injected into a carrier stream which flows to a detector. The injection is accomplished by the use of a rotary valve. The carrier stream flowrate is maintained by a precision peristaltic pump. The sample "plug" is injected into the carrier stream and a transient signal appears at the detector.

The flow injection apparatus used in this study is a Lachat rotary valve model 1000 - 600. This is a electro-mechanical valve that is triggered automatically by TTL pulses generated in the central processing unit of the ICP-MS computer. Liquid flow is controlled by a peristaltic pump (Gilson Minipuls - 2). The detector used is a Sciex Elan model 250 ICP-MS system connected to a stand alone personal computer for data handling.

Flow injection produces a transient signal. Transient signal measurement is accomplished by using a special transient measurement program in the Elan software. Originally this software was devised for an electro-thermal vaporization device. Utilization for the flow injection apparatus was quite straight forward, using the various ramp voltage timing sequences for the rotary valve. Automatic triggering of the data acquisition system is also possible using this software program. This allows for very reproducible results when measuring several peaks.

In addition to controlled triggering, appropriate dwell times must be used in order to obtain good precision. Dwell time is the length of time the computer is resident on a selected mass peak while acquiring data. Optimum dwell time will vary depending on the number of peaks and the transient signal duration. For single element analysis, experiments have shown that a 300 ms dwell time is optimum. At this dwell time the precision obtained after the measurement of seven different injections of a 100 ug/l aqueous Pb standard was approximately 3% R.S.D.

The residence time of a transient signal produced by flow injection will vary depending on the extent of dispersion. There are two modes of physical dispersion: convection, and radial molecular diffusion. The effects of each of these depend on a number of key experimental parameters (carrier stream flowrate, tubing size from the injector unit to the detector and sample loop volume). Various response curves have been produced for each of these parameters.

Once a set of response curves has been created, this information can be used to find optimum parameters. This will enable the utilization of dilution caused by dispersion to minimize matrix effects.

After optimising this particular system, a significant reduction in matrix effects was observed compared with continuous nebulization. However, the detection limits for the analytes investigated degraded by a factor of 5. Detection limits will vary with the extent of dispersion.

With conventional nebulizers, samples with high amounts of
dissolved solids (>1%) form oxides in the plasma and tend to
"clog" the sampling orifice of the ICP-MS. Experiments in this
study have shown that using flow injection to introduce high
dissolved solids solutions produces far less oxide. Therefore
orifice "clogging" is substantially reduced.

At present trace analysis of uranium in water, soils, vegetation,
air, and dustfall is done by conventional ICP-MS. Trace uranium
analysis in an industrial waste matrix poses a problem for
conventional ICP-MS due to the potential of high dissolved solids
in the matrix. Preliminary work on the development of a method to
estimate the concentrations of uranium by flow injection ICP-MS is
in progress. Initial results indicate that minimum matrix effects
are seen, and that the data are precise and accurate.

Flow injection in general is very versatile and offers a number of
in-line sample pretreatment processes. Preconcentration is one of
them (11). With an ever increasing demand to gain lower detection
limits, preconcentration with flow injection ICP-MS offers a
potential of detection power in the parts per quadrillion range.

Sample speciation studies are also possible. Separation can also
be accomplished in-line with a flow injection unit.

REFERENCES:

1.   Powell, M.J., Boomer, D.W., McVicars, R.J., Anal. Chem.,
      1986, 58, 2864-2867.

2.   Boomer, D.W., Powell, M.J., Salin, E.D., Sing, R.L.A.,
      Anal. Chem., 1986, 54, 975-976.

3.   Gray, A.L., Analyst, 1985, 110, 551.

4.   Hall, G.E.M., Park, Q., Boomer, D.W., Powell, M.J., JAAS,
      1988, 3 (in press).

5.   Ranger, C.B., Anal. Chem., 1981, 51, 20a-30a.

6.   Ruzicka, J., Hansen, E.H., Analtyica Chimica Acta, 1978, 99,
      37-76.

7.   Ruzicka, J., Anal. Chem., 1983, 55, 1041a-1053a.

8.   Houk, E.A., Thompson, J.J., Biomed. Mass Spectrom., 1983, 10,
      107.

9.   Thompson, J.J., Houk, R.S., Anal. Chemn., 1986, 58, 2541.

10.   Ebdon, L., Dean, J.R., Crews, H.M., Massey, R.C., JAAS, 1988,
      3, 349-354.

11.   Hartenstein, S.D., Ruzicka, J., Christen, G.D., Anal. Chem.,
      1985, 57, 21-25.

INVESTIGATION OF THE <u>IN SITU</u> ACETYLATION PROCESS - DETERMINATION OF ENVIRONMENTALLY SIGNIFICANT CHLORINATED MONO AND DIHYDRIC PHENOLS IN AQUEOUS MATRICES USING DUAL CAPILLARY COLUMN GAS CHROMATOGRAPHY

R. Lega, O. Meresz and M. Savu, Ministry of the Environment, Laboratory Services Branch, Trace Organics Section, 125 Resources Road, Rexdale, Ontario, Canada M9W 5L1

Chlorophenols constitute a group of compounds with a long history of environmental problems. These problems started to occur at the turn of the century with the general introduction of chlorine-disinfection of public water supplies. The severe taste and odour problems were traced to chlorinated phenols and to phenols in the raw water, thus analysis of "phenolics" in the source waters is probably the earliest example of environmental monitoring for organic compounds at the trace levels. Later, wood pulp bleaching produced and introduced a wide variety of chlorinated phenolic compounds directly to the aquatic environment. These substances have very high pollution potentials owing to their fish toxicity, bioaccumulation and fish tainting properties. Other sources of chlorophenols are penta chlorophenol-based wood preservatives and pesticides synthesized from chlorinated phenols.

The objective of the present work was to develop a gas chromatographic analytical scan for a wide range of chlorophenolic compounds that can occur in the environment, including derivatives of dihydric phenols such as catechol and its monomethyl ether guaiacol.

Since phenolic compounds, in general, are notorious for causing difficulties in gas chromatography, a derivatization step had to be considered. For this purpose, <u>in situ</u> acetylation was selected as the technique of choice. This reaction is receiving increasing attention in organic trace analysis, as it is an elegant and simple technique that in one step achieves derivatization for gas chromatographic analysis and greatly improves recoveries of phenolic compounds from aqueous matrices.

A systematic study of the in-situ acetylation reaction was undertaken using selected phenols, and later the full range of analytes proposed for the scan. The full range of phenolic compounds is shown in Table 1.

TABLE 1. LIST OF CHLORINATED PHENOLIC COMPOUNDS INVESTIGATED

Chlorophenols

1.	Phenol	12.	2,4,6-Trichlorophenol
2.	2-Chlorophenol	13.	2,3,6-Trichlorophenol
3.	3-Chlorophenol	14.	2,3,5-Trichlorophenol
4.	4-Chlorophenol	15.	2,4,5-Trichlorophenol
5.	2-Chloro-5-Methylphenol	16.	2,3,4-Trichlorophenol
6.	2,6-Dichlorophenol	17.	3,4,5-Trichlorophenol
7.	4-Chloro-3-Methylphenol	18.	2,3,5,6-Tetrachlorophenol
8.	3,5-Dichlorophenol	19.	2,3,4,6-Tetrachlorophenol
9.	2,3-Dichlorophenol	20.	2,3,4,5-Tetrachlorophenol
10.	2,4-Dichlorophenol	21.	Pentachlorophenol
11.	3,4-Dichlorophenol		

Chloroguaiacols

22.	4-Chloroguaiacol	25.	4,5,6-Trichloroguaiacol
23.	4.5-Dichloroguaiacol	26.	3,4,5,6-Tetrachloroguaiacol
24.	3,4,5-Trichloroguaiacol		

Chlorocatechols

27.	4-Chlorocatechol	29.	3,4,5-Trichlorocatechol
28.	4,5-Dichlorocatechol	30.	Tetrachlorocatechol

Others (non Primary Target)

31.	3,4,5-Trichlorosyringol	33.	Guaiacol
32.	5,6-Dichlorovanillin	34	Syringaldehyde

During the study, the following variations were explored:
1. Running the acetylation without and with bicarbonate buffer.
2. Addition of extraction solvent before or after the main acetylation reaction.
3. Addition of 2.5 or 5.0 mL acetic anhydride to 500 mL sample.
4. Addition of acetic anhydride in one or two portions.
5. Absence or presence of a catalyst.
6. Addition of different catalysts:
   a) 4-(dimethylamino)pyridine
   b) pyridine
   c) eucalyptol
7. Single/multiple extraction.
8. Direct/stepwise evaporation of extract.

The following observations were made:
1. In the absence of bicarbonate buffer, acetylation does not take place to a practically acceptable extent.
2. The addition of extraction solvent (methylene chloride) after 15 min. reaction time (and not before the addition of acetic anhydride) resulted in little changes in recoveries with the exception of guaiacols. The recovery of tetrachloroguaiacol doubled, from 41 to 82% (average of eight experiments).
3. Doubling the quantity of acetic anhydride (from 2.5 to 5.0 mL) had little effect on recoveries.
4. The addition of acetic anhydride (5.0 mL) in two 2.5 mL portions made little difference in recoveries.
5. Addition of catalyst generally increases recoveries especially for those components which are difficult to acetylate (such as guaiacols, syringaldehyde).
6. Pyridine and 4-(dimethytamino) pyridine showed comparable catalytic effects while eucalyptol had a very slight positive effect on the reaction.
7. Multiple extraction of the reaction mixture gives slightly higher recoveries when the same total quantity of solvent is used but the difference does not justify the effort required.
8. Stepwise evaporation of dried extracts gave slightly lower but more consistent recoveries.

Sample extracts were analysed on a Hewlett Packard 5730A Gas Chromatograph equipped with dual flame ionization detector and dual capillary column (30m, 0.25mm 10, 0.25u coating DB17 and DB1).

The method detection levels of all the compounds were calculated according to the Ontario Ministry of the Environment guidelines, and were found to be in the range 2-5 ug/L.

DP11

ROBUSTNESS OF SIMPLE HYPOTHESIS TESTING METHODS WITH CENSORED ENVIRONMENTAL QUALITY DATA

E.E. Creese

Creese Environmental Consulting,
P.0. Box 91, Waterloo, Ontario
N2J 3Z6

INTRODUCTION

Frequently when a chemical parameters of environmental concern (e.g. chlorinated organics or heavy metals) is measured, a sample will contain some observations below the analytical detection limit. Such data is termed *censored* data set.

Previous studies (Gilliom & Helsel, 1986; Helsel & Gilliom, 1986) have shown that, of the methods that they investigated, log-probability regression to the curve of a normal distribution was the most robust for estimating population parameters such as the mean and standard deviation of parent populations of environmental data. This work takes as a starting point the assumption that the lognormal distribution is the most likely parent distribution of chemical water quality data. It endeavors to assess the reliability of simple hypothesis testing, such as the z test or the t test, when population or sample parameters are estimated by log-probability regression.

The basic procedure is to generate a large number of simulated samples, all with a specified number of observations. Then censoring is performed at a certain defined cut-off point. The mean and variance of the population (or sample, depending on the method) are estimated by log-probability regression. Then, a hypothesis test is performed using the estimated mean and variance to determine if the sample mean can be inferred to be equal to the population mean. Since the population mean is known, which, of course, is the whole point of using simulated data, one can say whether or not the statistical test failed. Such a failure, that of rejecting a true null hypothesis, is termed a type I error. This work involves the use of Monte Carlo methods to compare to compare nominal type I error rates to actual type I error rates. At present, several regression methods are being compared, but only one is reported on here. The work is still in progress. For this reason, methods are the main topic of discussion in this paper. Only preliminary results are available.

METHODS

The work is being carried out on a Macintosh Plus microcomputer with a 20 megabyte hard drive. Random number generation and statistical routines are performed with the Systat application. All the Systat calculations are done in double precision, an automatic feature of most Systat routines. Iterative Systat programs are assembled by a Hypercard program and submitted for batch execution to Systat using the Macromaker utility. Macromaker returns control to Hypercard, which then composes the next set of Systat programs, and so on.

Generation of random numbers

The algorithm used by Systat for the generation of random numbers is given in Wichman & Hill (1982). It has a cycle length of 2.78×10^{13}. On opening the Systat DATA module, the random number generator always starts at the same point in the cycle. It is possible to provide a seed number between 1 and 30,000 that can be entered to make the generator start at a different point. This, in effect, reduces the cycle to 30,000, supposing, of course, that the choice of the seed is random. For this reason, a batch of 10,000 random numbers with the required probability distribution was produced initially in a single session in the DATA

module. When, say, another 10,000 are required, the Systat DATA module will be opened, 20,000 random numbers will be produced, discarding the first 10,000.

Generation of random samples

As mentioned above, a set of 10,000 lognormally distributed random numbers, x_i, was produced. The population median was set to $v_x = 1$ and the coefficient of variation to $cv_x = 1$. The first step in generating the numbers was to generate the corresponding normally distributed numbers, y_i, where $x_i = \exp[y_i]$. Normal random samples were simulated using the equation:

$$y_i = \mu_y + \sigma_y \, \varepsilon_i$$

In this equation, ε is the error term, which is a normally distributed random variate with a mean of 0 and a variance of 1. It was provided by the Systat random number generator. It is apparant that:

$$\mu_y = v_y = \ln[v_x] = 0.$$

The relationship between σ_y and cv_x is given by Aitchison & Brown (1957):

$$cv_x^2 = \exp[\sigma_y^2] - 1.$$

Samples of sample size $n = 10$ were abstracted from the set thus generated, the first 10 numbers becoming the first sample, the next 10 becoming the second sample, etc.

Censoring

Four censoring points were chosen to correspond to the 20th, 40th, 60th and 80th percentiles of the parent population. These correspond to four hypothetical detection limits, x_D, of chemical analysis. They are calculated from the normal distribution as follows:

$$x_D = \exp[\mu_y + z[p] \, \sigma_y] \quad | \, p \, \varepsilon \, \{.2,.4,.6,.8\}$$

Table 1. Detection limits, x_D, corresponding to the 20th, 40th, 60th and 80th percentiles of a lognormally distributed quantity with median value and coefficient of variation both equal to 1.

Percentile	x_D
20	0.496
40	0.810
60	1.235
80	2.015

Baseline Check

The set of 10,000 random numbers generated were tested for compliance with the original population parameters that defined it. This was done to ensure that the computer programs were indeed written and executed according to plan, in other words, to make sure that everything was done right. To accomplish this, 1,000 samples of $n = 10$ were tested in turn by a Student's t test in Systat module, STATS, against the true hypothesis that $\mu_y = 0$. Unlike SAS, or SPSS, which determine the probabilies corresponding to t by interpolating from a table, Systat calculates the probability, resulting in greater accuracy. The algorithm for this calculation is decribed in Lund & Lund (1983).

The results of this check are shown in Table 2. At a nominal type I error rate of $\alpha = 5\%$, it is to be expected that the t test will fail 5% of the time. Table 2 shows that the actual type I error was $a = 4.9\%$, which is well within the 99.9% confidence limits of the nominal type I

error rate. The 99.9% confidence interval of α was determined from the following expression:

$$\alpha \pm z[.9995]\, (\alpha\,(1-\alpha)\,/\,m)^{0.5}$$

where m is the number of tests, in this case, 1,000.

Table 2. Results of 1,000 t tests performed on simulated environmental quality data. α is the nominal type I error rate and a is the actual type I error rate.

α	a	99.9% confidence interval of α
5%	4.9%	4.8% - 5.2%

Simulation Runs

In these runs, simulated samples of n = 10 were ordered by rank. Then they were censored at one of the four points listed above in Table 1. Normal scores, z, were then computed for each of the remaining sample observations by:

$$z_i = z[R_i\,/\,(n+1)],$$

where R_i is the rank of the observation. Regression of $\ln[x]$ on z was then done according to the model,

$$\ln[x] = m_y + s_y\,z,$$

to obtain estimates of the population mean, μ_y, and standard deviation, σ_y. At higher levels of censoring, some samples had to be discarded, since regression could not be performed if less than two sample observations remained above x_D. After regression, hypothesis testing was done, choosing as the null hypothesis that the sample mean is equal to the population mean. Type I errors were counted, and at the end of each run, a, the actual type I error rate was calculated.

RESULTS

To date, very few data generating runs have been completed. So far hypothesis testing has been limited to a z test. A confidence interval for the appropriate probability, p, is constructed about the estimate of population mean, thus:

$$m_y \pm z[p]\,s_y\,/\,\sqrt{n} \mid p = 1 - \alpha\,/\,2$$

A type I error is indicated if the true population mean, μ_y, falls outside the confidence interval.

Table 3. Results of z tests on 500 samples of sample number n = 10, performed on simulated environmental quality data. The nominal type I error rate is $\alpha = 5\%$. a is the actual type I error rate, m is the number of z tests actually performed.

censoring level	a	m	95% confidence interval of α
0%	4.8%	500	4.0% - 6.0%
20%	6.2%	500	4.0% - 6.0%
40%	12.8%	499	4.0% - 6.0%
60%	26.7%	475	4.0% - 6.0%
80%	50.5%	325	3.8% - 6.2%

The trend seen in Table 3 is of course what would be expected. The power of the z test to distinguish the correct hypothesis decreases with the level of censoring. It can be seen that at 80% censoring, the z test has lost all discriminating power. It is basically a 50:50 chance whether or not it picks the correct hypothesis.

DISCUSSION

More preliminary experimentation is required before production of tables can begin. It will be necessary to know, for example, whether the absolute number as well as the proportion of observations above detection influences the actual type I error rate.

The ultimate goal of this work is to generate tables so that one could perform a standard hypothesis test at a given nominal type I error rate and then look up what the actual type I error is. Alternatively, one could decide, given the degree of censoring, what nominal type I error to test a hypothesis at in order to obtain the desired actual type I error probability. Such tables would be of great use to practising environmental scientists.

REFERENCES

Aitchison, J. & J.A.C. Brown. 1957. *The Lognormal Distribution with special reference to its uses in economics*. Cambridge University Press.

Gilliom, R.J. & D.R. Helsel. 1986. Estimation of distributional parameters for censored trace level water quality data. I. Estimation techniques. *Water Resources Research, 22(2):* 135-146.

Helsel, D.R. & R.J. Gilliom. 1986. Estimation of distributional parameters for censored trace level water quality data. 2. Verification and applications. *Water Resources Research, 22(2):* 147-155.

Lund, R.E. & J.R. Lund. 1983. Probabilities and upper quantiles for the studentized range. Algorithm AS 190. *Applied Statistics, 312:* 204-210.

Wichman, B.A. & I.D. Hill. 1982. An efficient and portable pseudo-random number generator. Algorithm AS 183. *Applied Statistics, 311:* 188-190.

AUTOMATION OF SOLID SUPPORTED REACTIONS BY ROBOTICS:

J.M. Rosenfeld[1] and E. Pevolinas[2].

1. Department of Pathology and 2. Department of Engineering Physics, McMaster University. Hamilton Ont. L8N-3Z5

Automation is a necessary development for environmental laboratories which more than any other organization are required to analyze numerous samples and under time pressure. These determinations are frequently made more complex by the need to both extract and derivatize the required analytes. The current methods are based on manual sample preparation including isolation and derivatization. Solid supported processes have been suggested as an approach to simplify the isolation phase of sample preparation of analytical methods. We have investigated solid supported reactions as a means to develop a sample preparation technique that is based totally on solid phase processes. Such processes, we believe are more readily amenable to automation. We selected robotics as the approach to automation because of the flexibility inherent in this technique. We considered such flexibility important because of the diverse and extensive problems encountered in most analytical laboratories. We have developed a prototype robotic system for incorporating such reactions into automated methods of analysis.

The system consists of a CRS plus lightweight robot (Burlington Ont.) and a peripheral work station. The peripheral has the performs the following functions. Solvents and buffer in 1-10 mL volumes are added to the reaction vessel via a nozzle fed by pumps. The nozzle is directed to the vessel by the robotic arm. Reagents are added my means of a solenoid activated syringe. Reaction mixtures consisting of a solid adsorbent and catalyst are stirred and heated at any one of 20 locations located above on 35 position multi point stirrer. The reaction mixture is liquid is removed from the solid/aqueous mixture by aspiration or by filtration of the liquid phases. The robotic arm moves the aspirating tube and the sample to the eluting/concentration positions that are on the remaining 15 positions in the multipoint stirrer. The derivatized analyte is eluted from the filtrate is finally concentrate to residue at these positions. All processes of the peripheral and manipulations of the arm are controlled by an XT clone.

The system was tested for the pentafluorobenzylation of pentachlorophenol with XAD-2 supported reaction as the model. Replicates of 6 analyses were carried out and with a relative standard deviation of 15%. Similar derivatizations performed manually had relative standard deviations of less than 10%. Both gave comparable yield. It is as yet unclear if the larger standard deviation is a function of the reaction or of the evaporation method.

INDICES LISTED FOR REFERENCE
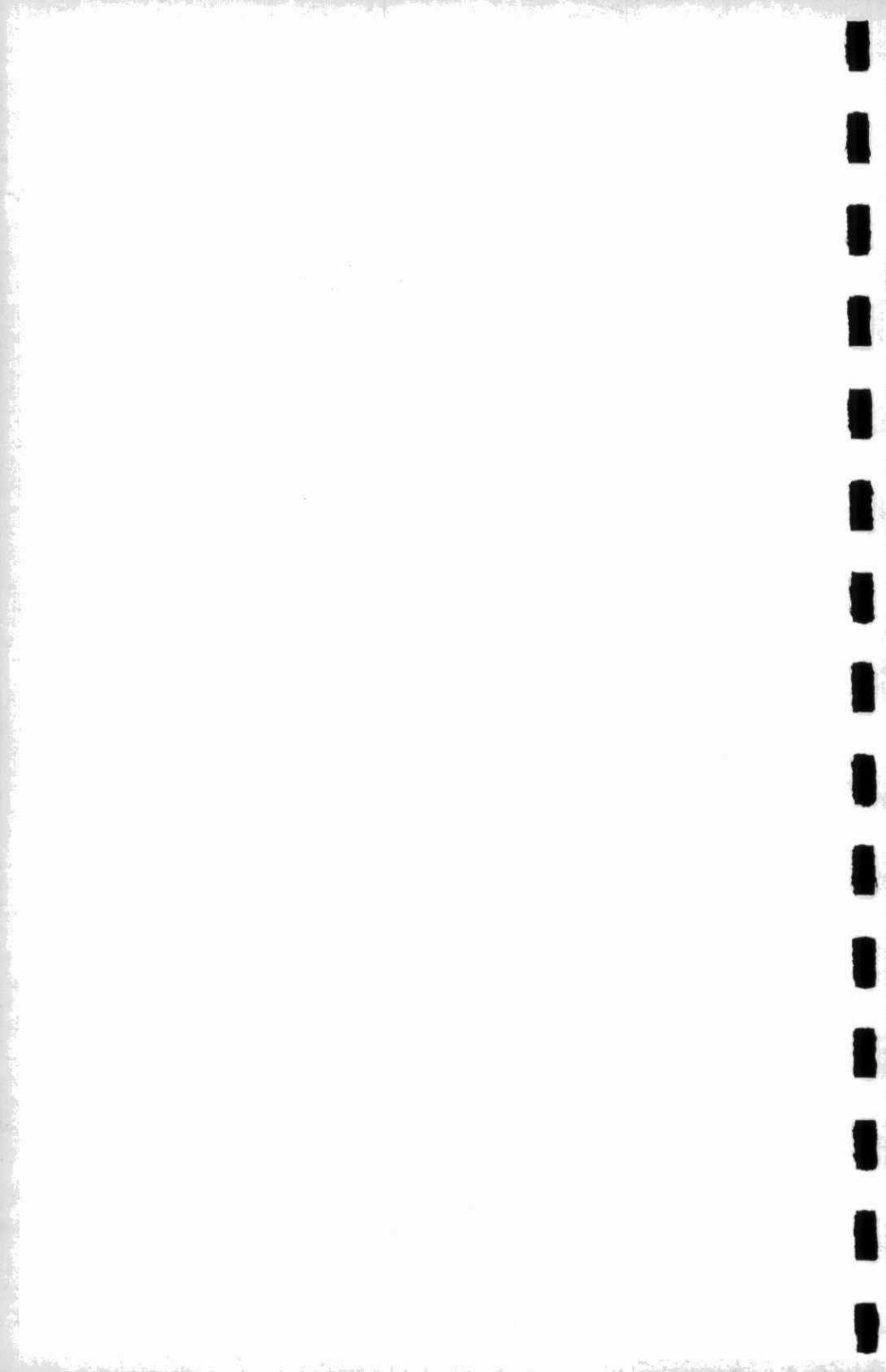
Abstract

SESSION A: AIR QUALITY RESEARCH

Oral Presentations

A1   Science and Policy: PhotoChemical Oxidants and Acid Bearing Species K. L. Demerjian, Atmospheric Science Research Center, State University of New York, Albany, New York, U. S. A.

A2   Relationship Between Forest Decline and Root Health in Ontario Sugar Maple C. Adams, M. Egyed and T. Hutchinson*, Dept. of Botany, University of Toronto, Toronto, Ontario.

A3   A Numerical Decline Index Rating System to Monitor Changes in Tree Condition of Hardwood Forest Species D. McLaughlin*, W. McIlveen, W. Gizyn, D. Corrigan, R. Pearson and R. Arnup, Air Resources Branch, Environment Ontario

A4   Investigation of Short-term Mutagenicity and Chemical Composition of Organic Solvent Extractable Fraction of Coke Oven Emission A. J. Horton*, N. Belson, K. Shaw and G. H. Thomas, Ontario Research Foundation, Clarkson, Ontario

A5   Quantitative Measurements of the Genetic Effects of Inhaled Carcinogens in Pulmonary Fibroblasts are Now Possible J. A. Heddle*, A. Bouch and J. D. Gingerich, Dept. of Biology, York University, Downsview, Ontario

A6   Sensitivity of Asthmatic Children to Air Pollution; D. Pengelly* and C. Goldsmith, McMaster University, Hamilton, Ontario

A7   Hazardous Contaminants in Ontario: Environmental Fate and Human Exposure D. Mackay* and S. Paterson, Institute for Environmental Studies, University of Toronto, Toronto, Ontario

Abstract

SESSION A: AIR QUALITY RESEARCH

Oral Presentations

Abstract

309

Abstract

SESSION A: AIR QUALITY RESEARCH

Poster Presentations

AP1 Stochastic Modelling of Dispersion from Single Elevated Sources E. Robertson and P. J. Barry, Atomic Energy of Canada Limited, Chalk River Nuclear Laboratories, Chalk River, Ontario

AP2 Feasibility Study for Assessing and Modelling Microclimatic Conditions on the Fonthill Kame (Phase 1) T. B. Shaw, Brock University, St. Catharines, Ontario

AP3 Critical Evaluation of Atmospheric Pollutant Parameterization from Satellite Imagery N. T. O'Neill, A. Royer and L. Hubert, Universite de Sherbrooke, Sherbrooke, Quebec, and J. Miller and J. Freemantle, CRESS, York University, Downsview', ', Ontario, and G. Austin and A. Davis, McGill

AP4 A 3-D Mesoscale Wind Field Model and its Application for Emergency Planning at Nuclear Power Plants in Ontario
 H. Sahota, P. K. Misra, R. Bloxam and D. Rhee, Air Resources Branch, Environment Ontario

AP5 The Results from a Meso-scale Model M. Niewiadomski, University of Toronto, Toronto, Ontario

AP6 Dose Response for Selected Environmental Air Pollutants: Results from a Study on Runners R. B. Urch, F. Silverman, P. Corey and R. J. Shephard, The Gage Research Institute, University of Toronto, Toronto, Ontario

Abstract

Abstract

Abstract

SESSION B: WATER QUALITY RESEARCH

Oral Presentations

B1   Aquatic Biology in the New Regulatory Framework K. Day, National Water Reseach Institute, Burlington, Ontario.

B2   Hypothesis Testing in Aquatic Toxicology: QSAR Relationships and Simple Kinetic Modelling L. S. McCarty*, University of Waterloo, Waterloo, Ontario, G. W. Ozburn and A. D. Smith, Lakehead University, Thunder Bay, Ontario.

B3   Variations in the Response of Fish Population Characteristics to Environmental Changes K. R. Munkittrick* and D. G. Dixon, Department of Biology, Universityof Waterloo, Waterloo, Ontario.

B4   An Examination of Chronic Toxicity of Thiocyanate to Freshwater Fish for the Development of a Water Quality Criterion D. G. Dixon, R. P. Lanno* and S. D. Kevan, University of Waterloo, Waterloo, Ontario.

B5   Potential Role of Polycyclic Aromatic Hydrocarbons in the Development of Liver Tumors in Fish from Polluted Sites of Lake Ontario G. M. Kirby, I. R. Smith, C. Thorn, H. W. Ferguson and M. A. Hayes*, Univeristy of Guelph, Guelph Ontario.

B6   Plant Bioassays for the Detection of Environmental Mutagens in an Aquatic Environment W. F. Grant, Department of Biology, York University, Downsview, Ontario.

B7   Effects of Temperature and Field Procedures on PCB Bioaccumulation in Elliptio Complanata A. Melkic* and Y. Rollin, Intergrated Explorations, Guelph, Ontario.

Abstract

SESSION B: WATER QUALITY RESEARCH

Oral Presentations

B8 Biomonitoring: Chemical Dependent Quantitative
Relationships for the Body Burdens of Organic
Chemicals in Aquatic Biomonitors F. Gobas*, R.
Russell and G. Haffner, Great Lakes Institute,
Univerisity of Windsor, Windsor, Ontario.

B9 Biomonitoring Protocols for Adult Aquatic Insects:
Seasonal Availability, Sample Size and Sensitivity
Z. E. Kovats and J. J. H. Ciborowski*, Dept. of
Biological Sciences, University of Windsor, Windsor,
Ontario.

B10 An Ecosystem Approach to the Monitoring of PCB's in
Pristine Ontario Lakes C. D. Metcalfe* and C. R.
Macdonald, Trent University, Peterborough, Ontario.

B11 Metal Contamination of Wetland Foodchains in the Bay
of Quinte, Ontario A. Crowder*, W. Dushenko and J.
Greig, Dept. of Biology, Queen's University,
Kingston, Ontario.

B12 An Overview of Aquatic Environmental Research in
Quebec M. Slivitsky, INRS-EAU, Ste. Foy, Quebec.

B13 Development of an Improved System for the Application
of Powdered Activated Carbon in Water Treatment
Plants H. Donison*, A. Benedek and J. J. Bancsi, Zenon
Environmental Inc., Burlington, Ontario.

B14 Municipal Utilization of Water Demand Management
Strategies in Ontario Municipalities R. D.
Kreutzwiser* and R. B. Feagan, Dept. of Geography,
University of Guelph, Ontario.

Abstract

SESSION B: WATER QUALITY RESEARCH

Oral Presentations

B15 A Preliminary Study to Determine the Feasibility of Medium Pressure Mercury Lamps for Disinfecting Low Quality Wastewaters G.E. Whitby and G. Sakamoto, Trojan Technologies Inc., London, Ontario, and G. Palamateer*, Envioronment Ontario.

B16 Characterization of the Fecal Indicator Bacterial Flora of Sanitary Sewage with Application to Identifying the Presence of Sanitary Waste in Storm Sewers P.L. Seyfried*, T. Bleier, Y. Xu and R. Harmandayan, University of Toronto, Toronto, Ontario.

B17 Landsat-5 TM Spectral Responses for Lakes Across Northeastern Ontario J.R. Pitblado, Geography Department, Laurentian University, Sudbury, Ontario.

B18 Relationship of Mercury Levels in Sportfish with Lake Sediment and Water Quality Variables C.D. Wren, B.A.R. Environmental, Quelph, Ontario.

B19 Trend Analysis Procedures for Water Quality Time Series A.I. McLeod*, and K.W. Hipel, McLeod-Hipel Associates Ltd., London, Ontario and B. Bodo, Environment Ontario.

B20 Use of a Bromobenzoate for Cross-Adaptation of Anaerobic Bacteria in Lake Ontario Sediments for Biodegradation of Chlorinated Aromatics M. Urbanek*, T. Strycek, R.C. Wyndham and M. Goldner, University of Toronto, Toronto, Ontario.

Abstract

SESSION B: WATER QUALITY RESEARCH

Poster Presentations

BP1 The Effects of Agricultural Drainage on Sediment and Water Quality Loadings W. E. Watt, Department of Civil Engineering, Queen's University, Kingston, Ontario.

BP2 WATQUAS 2.0: An Expert System for the Water Quality Assessment of Ontario Rivers W. C. Allison and T. E. Unny, Department of Civil Engineering, University of Waterloo, Waterloo, Ontario, and L. Logan, Environment Ontario.

BP3 Geochemical Characterization, Size Fractionation and Bioavailability of Trace Metal Particulate Associations in the Don River L. Warren and A. P. Zimmerman, Department of Zoology, University of Toronto, Toronto, Ontario.

BP4 The Investigation, Evaluation and Recommendation of Biomonitoring Organisms for Procedures Development for Environmental Monitoring C. A. Jefferson, Curry-Jefferson Environmental Services, Port Perry, Ontario.

BP5 The Ontario Inland Lakes Program and Management of Blue-Green Algae: Three Whole Lake Treatments in 1988 H. Vandermeulen and K. H. Nicholls, Water Resources Branch, Environment Ontario.

BP6 Characterization of the Grazing Fauna Within Five Softwater Lakes With Respect to Accumulations of Metaphytic Filamentous Algae P. M Stokes, E. T. Howell and R. L. France, Institute for Environmental Studies, University of Toronto, Toronto, Ontario.

Abstract

SESSION B: WATER QUALITY RESEARCH

Poster Presentations

BP7 Sedimentary Chrysophycean Cyst Assemblages as Paleoindicators in Acid Sensitive Lakes M. Rybak and I. Rybak, ARECO Canada Inc., Ottawa, Ontario, and K. Nicholls, Environment Ontario.

BP8 Factors Regulating Contaminant Levels In Forage Fish Species C. E. Herbert and G. D. Haffner, Great Lakes Institute, University of Windsor, Windsor, Ontario.

BP9 The Isotopic Composition of Upland Forest Soil Sulphate D. R. Van Stempvoort and P. Fritz, Department of Earth Science, University of Waterloo, Waterloo, Ontario.

BP10 Recent Trends and Historical Changes in Water Quality of Lake Muskoka M. Rybak and I. Rybak, ARECO Canada Inc., Ottawa, Ontario, and K. Nicholls, Environment Ontario.

BP11 In-Situ Determination of Fecal Indicator Bacterial Survival in Agriculturally-Impacted Watersheds M. J. Walters, Lake Simcoe Region Conservation Authority, Newmarket, Ontario.

BP12 Development of an Acute and Chronic Sediment Bioassay Protocol Using Larval Mayflies and Juvenile Fathead Minnows G. Krantzberg and R. Pope, University of Toronto, Toronto, Ontario.

BP13 Three Hour Pulse Exposure of Potassium Thiocyanate to Rainbow Trout Eggs Before and After Water Hardening S. Kevan And G. Dixon, University of Waterloo, Waterloo, Ontario.

317

Abstract

SESSION C: LIQUID AND SOLID WASTE RESEARCH

Oral Presentations

C1 An Overview of Hydrogeological Aspects of Waste Disposal: Research Results and Implications J. Cherry, Waterloo Centre for Groundwater Research, University of Waterloo, Waterloo, Ontario.

C2 Immiscible Liquids and Vapours in Soil: Recent Experiments on Transport and Control G. Farquhar*, R. Bensen, D. Graham, E. McBean and B. Stickney, Dept. of Civil Eng., University of Waterloo, Waterloo, Ontario.

C3 Effects of Increasing Amounts of Non-polar Organic Liquids in Domestic Waste Leachate on the Hydraulic Conductivity of Clay Liners in Southern Ontario F. Fernandez* and R. M. Quigley, University of Western Ontario, London, Ontario.

C4 Technology Review: Biological Treatment of Hazardous Landfill Leachates J. Fein*, and P. Yu, Diversified Research Laboratories Ltd., Toronto, Ontario.

C5 Phase Partitioning Kinetics at Industrial Waste Land Treatment Sites D. Hockley and W. J. Snodgrass*, Beak Consultants, Toronto, Ontario.

C6 Preliminary Assessment of a Microfiltration/Reverse Osmosis Process for the Treatment of Landfill Leachate T. A. Krug* and S. McDougall, Zenon Environmental Inc., Burlington, Ontario.

C7 Anaerobic Treatment of Landfill Leachate G. P. Vicevic*, B. J. Forrestal and A. Stevenson, Ontario Research Foundation, Clarkson, Ontario.

Abstract

319

Abstract

SESSION C: LIQUID AND SOLID WASTE RESEARCH

Oral Presentations

C15 An Alternative to Incineration of Biomedical Waste: Hammermill/Chemical Decontamination J. Manuel, Waste Management Branch, Environment Ontario.

C16 Erosion of Landfill Covers J. Cuthill*, Department of Land Resource Science, University of Guelph, Guelph, Ontario, and K. McKague, Ecologistics Ltd., Waterloo, Ontario.

C17 Development of Backfill and Construction Application Guidelines for Ontario M. Kelleher* and B. Whiffin, CANVIRO Consultants, Mississauga, Ontario.

C18 Panel Discussion: Stemming the Rising Tide of Waste Moderator: D. Mackay, University of Toronto

Abstract

SESSION C: LIQUID AND SOLID WASTE RESEARCH

Poster Presentations

CP1 Retractable Composite Absorbents for Environmental Clean-up B. Gillies, E. Stubley, I. Treurnicht and L. Read, EcoPlastics Ltd. Willowdale, Ontario and O. Meresz, Laboratory Services Branch, Environment Ontario.

CP2 Treatment and Disposal of Hauled Sewage Under ', 'Part VII, Environmental Protection Act J. L. Smith, Oliver, Mangione, McCalla & Associates Limited, Nepean, Ontario.

CP3 Factors Affecting the Concentration of Metal Ions in Municipal Refuse Leachate G. Kosta, S. Pirani and D. Kirk, Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario.

CP4 Slow Rate Infiltration Land Treatment and Recirculation of Landfill Leachate in Ontario R. A. McBride, A. M. Gordon, P. H. Groenevelt, T. J. Gillespie and L. J. Evans, Departments of Land Resource Science and Environmental Biology, University of Guelph, Guelph, Ontario.

CP5 Establishing Vegetation on Erosion-prone Landfill Slopes in Ontario, Year Two T. W. Hilditch and C. P. Hughes, Gartner-Lee Ltd. , Markham, Ontario.

CP6 Evaluating Groundwater Velocity in a Low-Permeability Fractured Shale K. S. Novakowski and J. A. Cherry, Centre for Groundwater Research, University of Waterloo, Waterloo, Ontario.

Abstract

Abstract

SESSION E: ENVIRONMENTAL ECONOMICS

Oral Presentations

E1 Understanding Environmental-Economic Integration
P. A. Victor, VHB Research Ltd. , Toronto, Ontario.

E2 Economic Valuation Disparities and Environmental
Policies J. L. Knetsch, Economics Department, Simon
Fraser University, Burnaby, British Columbia.

E3 The Physco-social Impacts of Exposure to
Environmental Contaminants in Ontario: A Feasibility
Study S. M. Taylor*, J. Frank, M. Haight, D. Streiner,
S. Walter and N. White, McMaster University,
Hamilton, Ontario.

E4 Economic Assessments of MISA Regulations for Direct
Industrial Dischargers in Ontario O. E. Salamon* and
J. A. Donnan, Policy and Planning Branch, Environment
Ontario.

E5 The Extra Strength Sewer Surcharge to Regulate
Industrial Sanitary Waste Discharges M. Fortin*,
Ecologistics, Waterloo, Ontario, G. Zudovs, CANVIRO,
and J. Donnan and G. Zegarac, Environment Ontario.

E6 A Study of the Economic Factors Relating to the
Implementation of Resource Recycling or Zero-
Discharge Technologies for Heavy Metal Generating
Industries in Canada B. Fleet*, J. Kassirer, T.
Burrell, T. Sanger, C. Small and B. Cardoza,
University of Toronto, Toronto, Ontario.

E7 Determinants of Participation in Solid Waste Source-
Separation Programs in High-Rise Apartment Buildings
V. W. Maclaren, Department of Geography, University
of Toronto, Toronto, Ontario.

Abstract

SESSION E: ENVIRONMENTAL ECONOMICS

Poster Presentations

EP1 The New Economics of Sustainable Development R. Z. Rivers, Water Planning and Management Branch, Canada Centre For Inland Waters, Environment Canada, Burlington, Ontario.

EP2 The Environmental Effects of Forestry Operations in Ontario: How Much Do We Know? J. A. Dunster, Federation of Ontario Naturalists, Toronto, Ontario.